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Stability Indicating HPLC Method for Quantification of Solifenacin Succinate & Tamsulosin Hydrochloride along with Its Impurities in Tablet Dosage Form

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Abstract

A novel stability-indicating RP-HPLC method was developed and validated for simultaneous determination of Solifenacin Succinate & Tamsulosin Hydrochloride and its impurities in tablet dosage form. The method was developed using L1 column with gradient using the mobile phase consist of solvent-A (pH = 6.6, phosphate buffer + 0.5% Triethylamine) and solvent-B (90% Acetonitrile). The eluted compounds were monitored at 225 nm. Solifenacin Succinate & Tamsulosin Hydrochloride was subjected to oxidative, acid, base, hydrolytic, thermal and photolytic stress conditions. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantitation, accuracy, precision and robustness. The limit of quantification results was ranged from 0.135 - 0.221 µg/mL for Solifenacin Succinate impurities and 0.043 - 0.090 µg/mL for Tamsulosin Hydrochloride impurities. This method is suitable for the estimation of impurities and assay of Solifenacin Succinate & Tamsulosin Hydrochloride in tablets dosage form.

Keywords

Solifenacin, Tamsulosin, RP-HPLC, Impurities, Method Validation

1. Introduction

Solifenacin Succinate & Tamsulosin Hydrochloride is available with the brand name of "VESOMNI" in the form of modified-release tablets with the dosage strength 6 mg/0.4 mg. Therapeutic indication of this brand is to treat moderate to severe storage symp-

toms like urgency, increased micturition frequency and voiding symptoms associated with benign prostatic hyperplasia (BPH) in men who are not adequately responding to treatment with monotherapy [1] [2] [3].

Solifenacin succinate (SOL) is a competitive muscarinic acetylcholine receptor antagonist. Chemically, Solifenacin succinate is butanedioic acid (3R)-1-azabicyclo [2.2.2] octan-3-yl(1S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate having an empirical formula of $C_{27}H_{32}N_2O_6$ and molecular weight of 480.5528 gms/mol.

Tamsulosin (TAM) is a selective antagonist at α1-A and α1-B adrenoceptors in the prostate, prostatic capsule, prostatic urethra and bladder neck. It brings about relaxation of prostatic and urethral smooth muscle. Chemically Tamsulosin is 5-[(2R)-2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl]-2-methoxybenzene-1-sulfonamide having an empirical formula of $C_{20}H_{28}N_2O_5S$ and molecular weight of 408.512 gms/mol.

Monograph for SOL drug substance is available only in European Pharmacopoeia [4]. TAM drug substance and capsules monograph are available in USP [5] [6] [7]. Literature survey reveals that HPLC, Semi micro HPLC, UPLC, UV and LC-MS/MS methods for SOS [8]-[15] and HPLC, LC-ESI-MS/MS and spectrophotometric methods [16]-[23] for TAM are available. However no method was reported for the estimation of SOL & TAM and their impurities by HPLC in any of combination dosage forms. Israel *et al*. [24] reported a method for the estimation of SOL & TAM, but not for the quantification of impurities.

The objective of this article is to present a stability-indicating method to estimate SOL & TAM and its related compounds. In the present work, a simple, fast and precise liquid chromatographic method was developed for the determination of SOL & TAM and its impurities. The chemical structures of SOL & TAM and their impurities are presented in **Figure 1**.

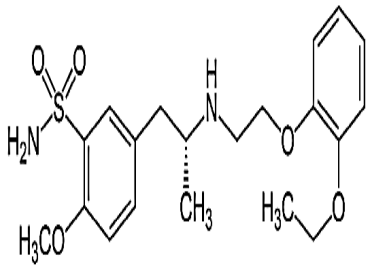
2. Experimental

2.1. Chemicals and Reagents

The purity of all chemicals used was above 99%. And standards of SOL (100%), TAM (100%) and their impurities namely SOL-1 (99.93%), SOL-2 (100%), SOL-3 (99.9%), TAM-1 (93.68%), TAM-2 (100%), TAM-3 (92%) and TAM-4 (89.4%) were supplied by Celltrion, South Korea. The HPLC gradient grade acetonitrile from J T Baker, and analytical grade ortho phosphoric acid, monobasic potassium phosphate and triethylamine were purchased from Sigma Aldrich. High purity water was prepared by using Milli-Q Plus water purification system (Millipore USA). Standard and Test samples were prepared in Acetonitrile and Milli-Q water in the ratio of 50:50 v/v as diluent.

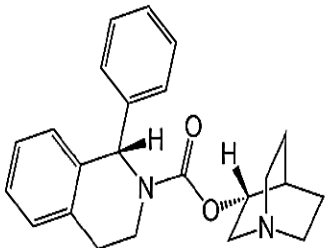
2.2. Equipment

Analysis was performed with an Agilent 1260 HPLC (Germany). HPLC system equipped with a quaternary solvent manager, sample manager, column-heating compartment, and Photodiode array detector. The output signal was monitored and processed using Chemstation software. Grant digital water bath was used for hydrolysis studies. Ther-



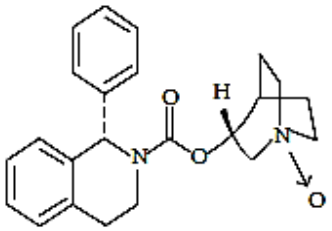
5-[(2R)-2-{[2-(2-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxybenzene-1-sulfonamide

A. Tamsulosin (TAM)



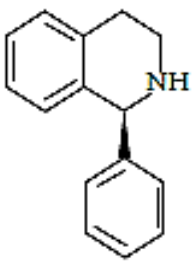
butanedioic acid (3R)-1-azabicyclo[2.2.2]octan-3-yl (1S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate

B. Solifenacin (SOL)



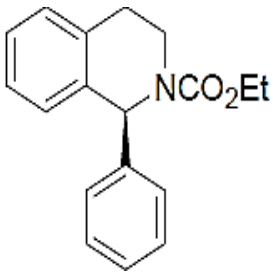
(1S)-3,4-dihydro-1-phenyl-2-(1H)-isoquinolinecarboxylic acid (3R)-1-azabicyclo[2.2.2]oct-3-yl ester N-oxide

C. SOL-1 /N-oxide



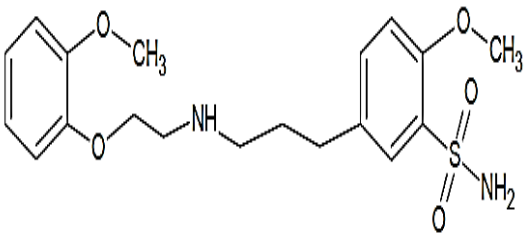
(1S)-1-phenyl-1, 2, 3, 4-tetrahydro isoquinoline

D. SOL-2 /S-IQL



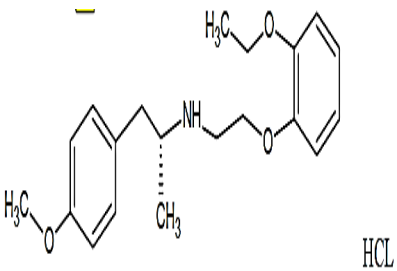
(S)-1,2,3,4-tetrahydro-1-phenylisoquinoline-2-carboxylic acid ethyl ester

E. SOL-3 /S-IQL-CARB



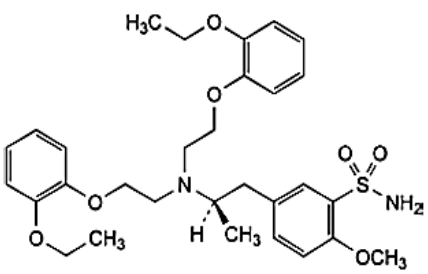
2-methoxy-5-(2R)-{[2-(2-methoxy phenoxy) ethyl] amino propyl} benzene sulphonamide

F. TAM-1 (EP-D)



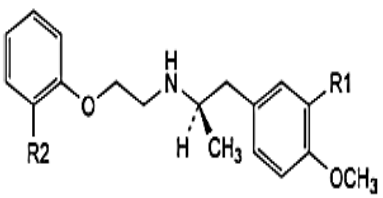
(2R)-N-[2-(2-ethoxy phenoxy) ethyl]-1-(4-methoxy phenyl) propan-2-amine hydrochloride

G. TAM-2 (EP-H)



5-[(2R)-2-[bis[2-(2-ethoxyphenoxy)ethyl]amino] propyl]-2-methoxy benzenesulfonamide

H. TAM-3 (EP-A)



R1=SO2-NH2, R2=H: 2-methoxy-5-[(2R)-2-[(2-phenoxyethyl)amino]propyl]benzenesulfonamide

I. TAM-4 (EP-C)

Figure 1. Chemical structure of solifenacin and tamsulosin, and their impurities (A. Tamsulosin (TAM); B. Solifenacin (SOL); C. SOL-1/N-Oxide; D. SOL-2/S-IQL; E. SOL-3/S-IQL-CARB; F. TAM-1 (EP-D); G. TAM-2 (EP-H); H. TAM-3 (EP-A) and I. TAM-4 (EP-C)).

mal stability studies were performed in a dry air oven (Thermo scientific). Mobile phase was degassed by ultra-sonication (Power sonic 420, Labtech) and filtered through

a 0.45 μm Nylon filter (PALL life sciences, USA).

2.3. Related Substances, Assay Standard and Sample Preparation

Preparation of Standard Solutions:

A stock solution of SOL and TAM (5000 $\mu g \cdot mL^{-1}$ of SOL and 332 $\mu g \cdot mL^{-1}$ of TAM) was prepared by dissolving appropriate amount of drugs in diluent (Milli-Q water and Acetonitrile 50:50 v/v). Working solutions of 24 $\mu g \cdot mL^{-1}$ of SOL and 1.6 $\mu g \cdot mL^{-1}$ of TAM, and 150 $\mu g \cdot mL^{-1}$ of SOL and 10 $\mu g \cdot mL^{-1}$ of TAM were prepared from the above stock solution for related substance and assay determination respectively. Individual impurity stock solutions were prepared in diluent.

Preparation of Sample Solution:

Tablet powder (6/0.4 mg tablets) equivalent to 120 mg of SOL (8 mg of TAM) drug was dissolved in diluent with sonication for 20 min to give a solution containing 4800 $\mu g \cdot mL^{-1}$ of SOL and 320 $\mu g \cdot mL^{-1}$ of TAM. The above solution was centrifuged at 4000 rpm for 5 minutes in order to eliminate insoluble excipients. The supernatant liquid was used for RS analysis. A solution with 144 $\mu g \cdot mL^{-1}$ of SOL and 10 $\mu g \cdot mL^{-1}$ of TAM were prepared by diluting supernatant solution for Assay analysis.

2.4. Chromatographic Conditions

The method was developed using Capcell Pak C18, MG, 150 × 4.6 mm; 5 μm column (Shiseido, Japan) with mobile phase containing a gradient mixture of solvent A and B. 20mM Monobasic potassium phosphate buffer with 0.5% triethylamine, pH adjusted to 6.6 with phosphoric acid was used as solvent-A and Milli-Q water and acetonitrile in the ratio 10:90 v/v; was used as solvent-B. The gradient program (T/%B) was set as 0/20, 10/40, 15/40, 22/70, 37/70, 38/20 and 45/20. The flow rate of the mobile phase was 0.9 $mL \cdot min^{-1}$. The column temperature was maintained at 30˚C and the chromatography was monitored at 225 nm. Injection volume was 10 μL.

3. Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The method was validated according to International Council for Harmonisation Q2 (R1) guidelines [25] for validation of analytical procedures in order to determine the specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness.

3.1. Solution Stability

The stability of SOL and TAM in solution for assay was determined by leaving test solutions of the sample and reference standards in tightly capped volumetric flasks at room temperature were assayed at 12 hrs intervals up to 24 hrs. The stability of SOL and TAM and their impurities in solution for related substance method was determined by leaving spiked sample solution in a tightly capped volumetric flask at room temperature for 24 hrs and measuring the amounts of the five impurities at every 12 hrs.

3.2. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed LC method for SOL and TAM was carried out in presence of its seven impurities. Stress studies were performed at the concentration 4800 $\mu g \cdot mL^{-1}$ of SOL and 320 $\mu g \cdot mL^{-1}$ of TAM on API in presence of excipients to show the stability indicating property of the method. The forced degradation was carried out on individual APIs to prove that degradents are not co-eluting with any of the known impurities and active molecule.

Intentional degradation was attempted to stress condition of UV light (200 watt hr/m^2), Sun-light (1.2 Mill lux hrs), heat (60˚C for 15 hr), acid (1 N HCl at 60˚C for 18 hr), base (1 N NaOH at 60˚C for 18 hr), water (at 60˚C for 24 hr) and oxidation (1.0% H_2O_2 at RT for 15 hr) for SOL and TAM to evaluate the ability of the proposed method to separate SOL and TAM from their degradation products. Peak purity test was carried out for SOL and TAM peaks by using PDA detector for stress samples.

3.3. Linearity

Linearity test solutions for SOL and TAM and their impurities were prepared by diluting stock solutions to required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% of the specification level 0.5% (LOQ, 0.10%, 0.25%, 0.50%, 0.75% and 1.0%). Linearity test solutions for the assay method were prepared from SOL and TAM stock solutions at 6 concentration levels from 50% to 200% of assay concentration (50%, 75%, 100%, 125%, 150% and 200% for SOL and TAM). The peak area versus concentration data was treated by least-squares linear regression analysis.

3.4. Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ for SOL and TAM and their impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting six individual preparations and calculated % RSD.

3.5. Accuracy

The accuracy of the assay method was evaluated in triplicate at five concentration levels 50%, 75%, 100%, 150% and 200% on tablets (6/0.4 mg). Standards of SOL, TAM and their impurities were spiked at different concentration levels namely LOQ, 0.25%, 0.5%, 0.75% and 1.0% with respect to their test concentration in triplicate and evaluated for accuracy of impurities. The percentage of recoveries for SOL, TAM and their impurities were calculated.

3.6. Precision

The precision of the method was verified by injecting six individual preparations of dosage form (SOL 6 mg and TAM 0.4 mg) spiked with 0.5% of its impurities. SOL im-

purities are spiked at 0.5% with respect to SOL concentration 4800 μg·mL^{-1} and TAM impurities are spiked at 0.5% with respect to TAM concentration 320 μg·mL^{-1}. % RSD of each impurity was calculated. Assay method precision was evaluated by carrying out six independent test preparations of SOL & TAM at 144 μg·mL^{-1} of SOL and 10 μg·mL^{-1} of TAM against qualified reference standard.

The same experiment was repeated on different day with different chromatographic system and different analyst to find variability.

3.7. Robustness

To determine the robustness of the method, experimental conditions were deliberately altered and the resolution between SOL and TAM impurities and tailing factors for SOL and TAM and their impurities were recorded. The effect of flow rate was evaluated at 0.7 and 1.1 mL·min^{-1} instead of 0.9 mL·min^{-1}. The effect of the column temperature was studied at 25˚C and 35˚C instead of 30˚C. The effect of pH of mobile phase buffer was studied by varying pH ± 0.1 units of method pH (6.6) keeping other mobile phase components constant.

4. Results

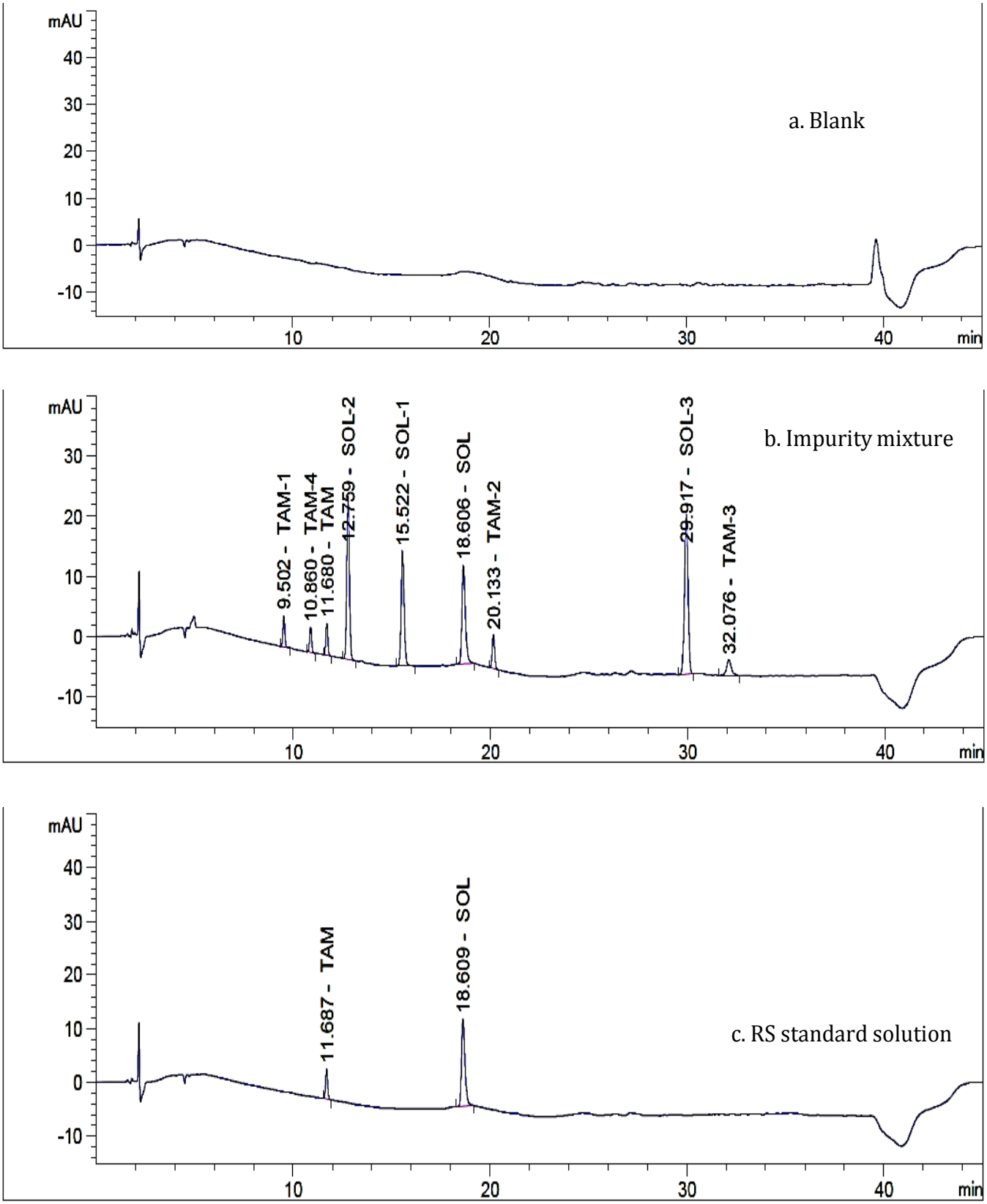
4.1. Method Development

The main target of the chromatographic method is to get the separation of impurities namely SOL-1, SOL-2 and SOL-3 of SOL & TAM-1, TAM-2, TAM-3 and TAM-4 of TAM and the degradation products generated during stress studies from the analyte peaks along with the actives.

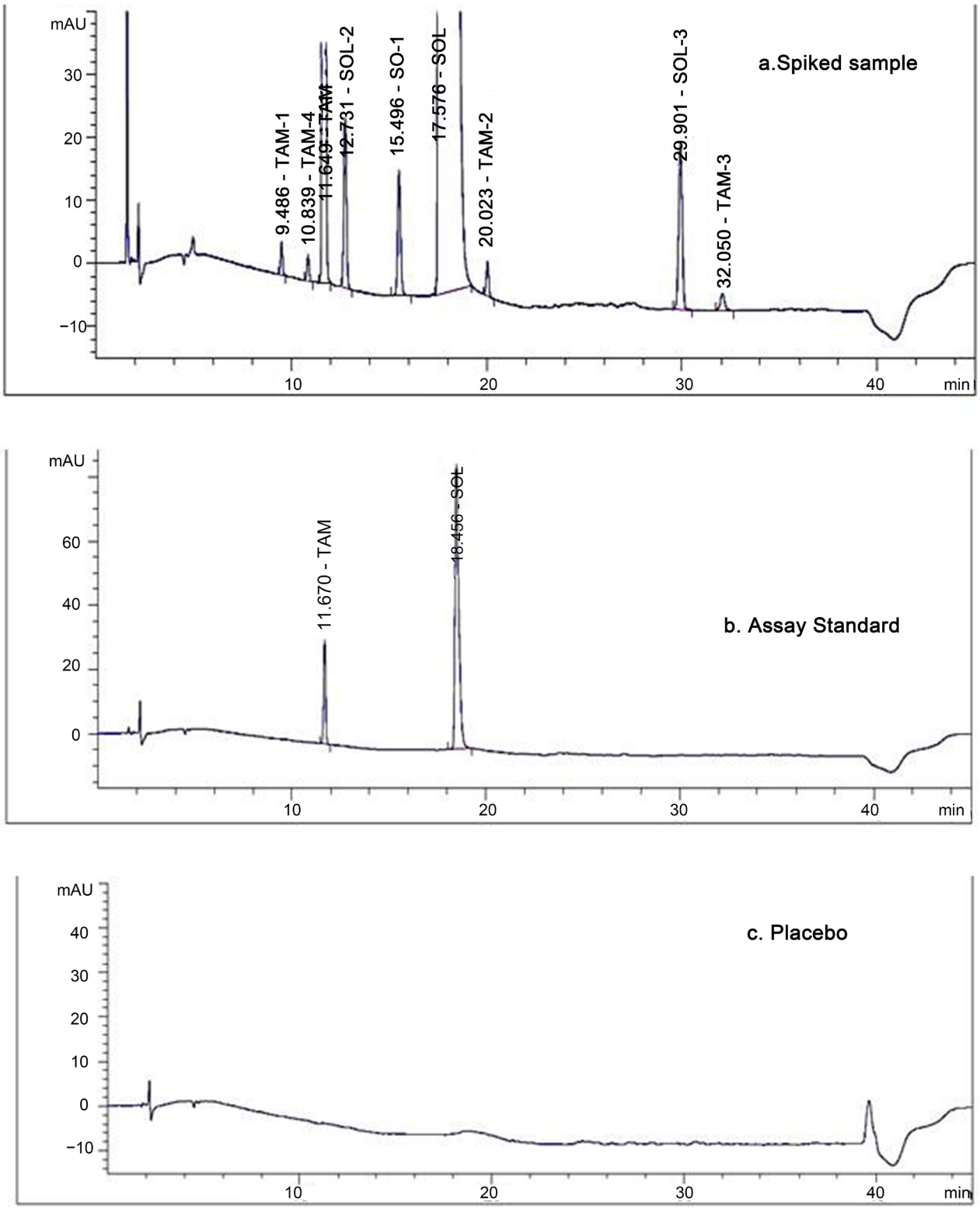
Chromatographic conditions with Capcell Pak C18, MG, 150 × 4.6 mm; 5 μm column and the mobile phase consists of solvent-A (20 mM Monobasic potassium phosphate buffer with 0.5% triethylamine, pH adjusted to 6.6 with phosphoric acid) and solvent-B (Milli-Q water and acetonitrile in the ratio of 10:90 v/v), with gradient program (T/%B) was set as 0/20, 10/40, 15/40, 22/70, 37/70, 38/20 and 45/20. The flow rate of the mobile phase was 0.9 mL·min^{-1}. The column temperature was maintained at 30˚C and the chromatography was monitored at 225 nm. Injection volume as 10 μL was suitable in separating SOL, TAM and its impurities from one other (**Figure 2(A)** & **Figure 2(B)**). Details of relative retention time, relative response factor, resolution and tailing factor values are summarized in **Table 1**.

4.2. Method Validation

Validation was performed on the developed analytical method for its acceptable performance to ensure suitability of intend purpose. The validation parameters like accuracy, precision, specificity, detection limit, quantification limit, linearity, range, ruggedness and robustness were executed and established method conditions to meet the requirements to execute the analysis of SOL and TAM combination dosage product. Under the specificity experiment samples were stressed at various stress conditions and analyzed along with unstressed samples. During the stress studies it was observed SOL


mAU
40
30
20
10
0
-10
10
20
30
40
min
a. Blank
b. Impurity mixture
9.502 - TAM-1
10.860 - TAM-4
11.680 - TAM
12.759 - SOL-2
15.522 - SOL-1
18.606 - SOL
20.133 - TAM-2
29.917 - SOL-3
32.076 - TAM-3
c. RS standard solution
11.687 - TAM
18.609 - SOL

(A)



(B)

Figure 2. (A) Typical chromatograms of solifenacin and tamsulosin at 225 nm (a. blank, b. impurity mixture and c. RS standard solution); (B) Typical chromatograms of solifenacin and tamsulosin at 225 nm (a. spiked sample, b. assay standard and c. placebo).

Table 1. Factor values of chromatographic method.

Compound	RT(min)	RRT*	RRF	Resolution	Tailing factor
TAM-1	9.486	0.81	0.94	N/A	1.00
TAM-4	10.839	0.93	0.82	6.90	1.01
TAM	11.649	1.0	1.0	4.02	0.99
SOL-2	12.731	0.72	1.30	4.96	1.03
SOL-1	15.496	0.88	1.03	10.65	1.08
SOL	17.576	1.0	1.0	3.68	1.15
TAM-2	20.023	1.72	1.19	4.70	1.00
SOL-3	29.901	1.70	1.73	34.32	0.98
TAM-3	32.050	2.75	1.12	6.12	1.13

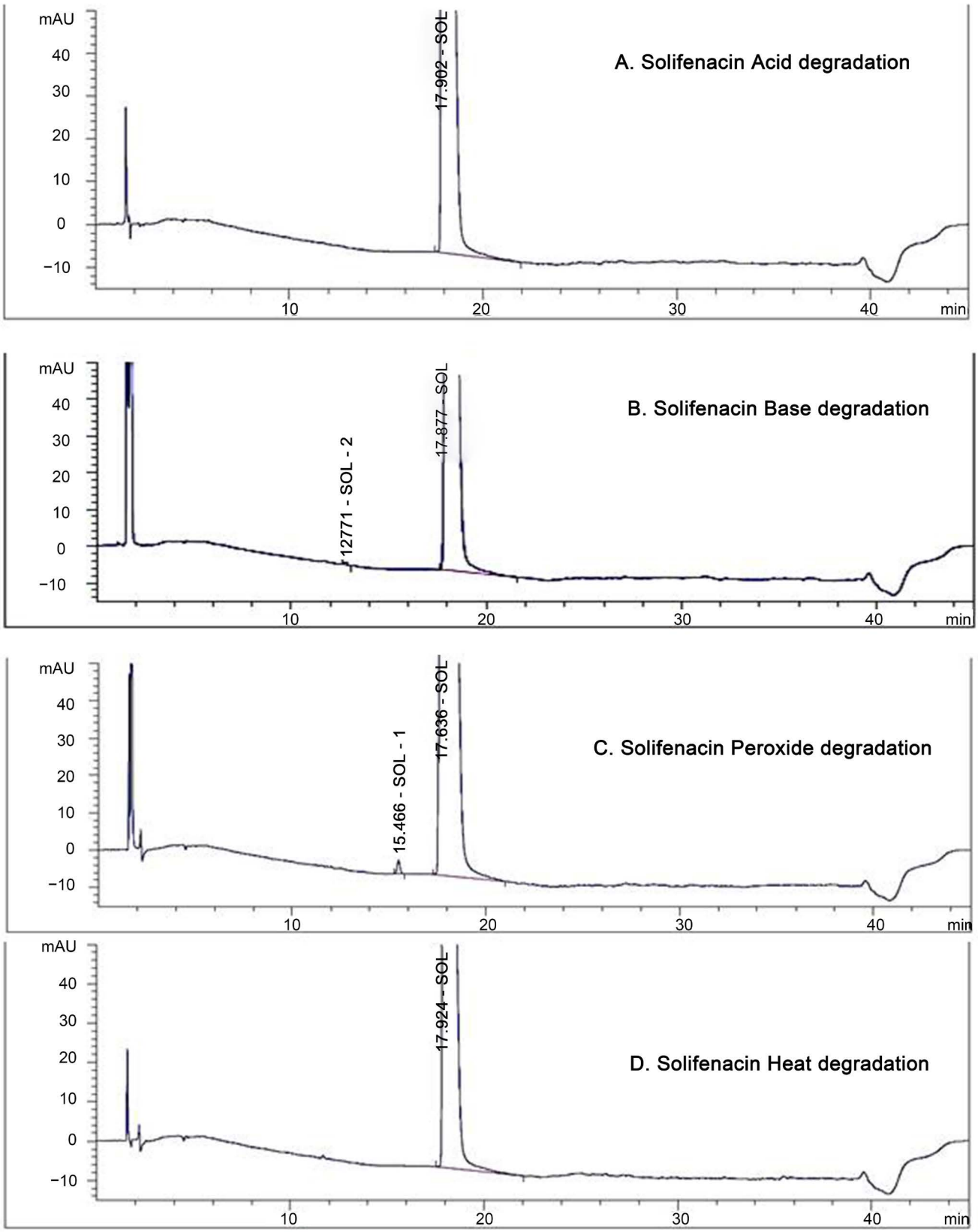
*Relative retention times (RRT) for TAM-1, TAM-2, TAM-3 and TAM-4 were calculated against the retention time (RT) of Tamsulosin and RRT of SOL-1, SOL-2 and SOL-3 were calculated against the retention time (RT) of Solifenacin.

and TAM were degraded significantly at particular conditions. SOL-1 impurity observed at oxidative (1% H_2O_2 at 25˚C for 15 h) condition (Figure 3(C)) and SOL-2 & TAM-4 impurities observed at base hydrolysis (1N NaOH at 60˚C for 18 h) condition (Figure 3(B) & Figure 4(B)). TAM-2 and one major unknown impurity at 25.985 min were observed at oxidative (1% H_2O_2 at 25˚C for 15 h) condition (Figure 4(C)). Results of forced degradation studies are reported in Table 2. Linearity, limit of quantification, limit of detection, precision, intermediate precision and precision at LOQ for SOL, TAM and its impurities were established and presented in Table 3. The percentage recovery of SOL, TAM and impurities in the estimation of impurities are presented in Table 4. Linearity, precision and intermediate precision results of SOL & TAM in the assay determination are presented in Table 5. The percentage recovery results for SOL & TAM in the assay determination are presented in Table 6.

5. Discussion

5.1. Method Development

The main target of the chromatographic method is to achieve the separation of impurities and the main components SOL & TAM with each other. A blended solution containing SOL impurities are spiked at 0.5% with respect to SOL concentration at 4800 $\mu g \cdot mL^{-1}$ and TAM impurities are spiked at 0.5% with respect to TAM concentration at 320 $\mu g \cdot mL^{-1}$ was prepared in diluent and used for the method's development. Initial experiments were performed with 20 mM monobasic potassium phosphate buffer (pH 6.5) as solvent-A and 90% acetonitrile as solvent-B with different gradient programs, using Inertsil ODS-3V (C-18, 150-mm 4.6-mm, 5 µm particles) column and found TAM-1, TAM-4, TAM peaks merged together and SOL-2, TAM-2 TAM-3 impurities response and peak shape found not good. Further checked with different stationery phase column Kromasil (C8, 150-mm 4.0-mm, 5 µm particles) and found TAM-1,


mAU
40
30
20
10
0
−10
17.902 - SOL
A. Solifenacin Acid degradation
10
20
30
40
min
mAU
40
30
20
10
0
−10
12771 - SOL - 2
17.877 - SOL
B. Solifenacin Base degradation
10
20
30
40
min
mAU
40
30
20
10
0
−10
15.466 - SOL - 1
17.636 - SOL
C. Solifenacin Peroxide degradation
10
20
30
40
min
mAU
40
30
20
10
0
−10
17.924 - SOL
D. Solifenacin Heat degradation
10
20
30
40
min

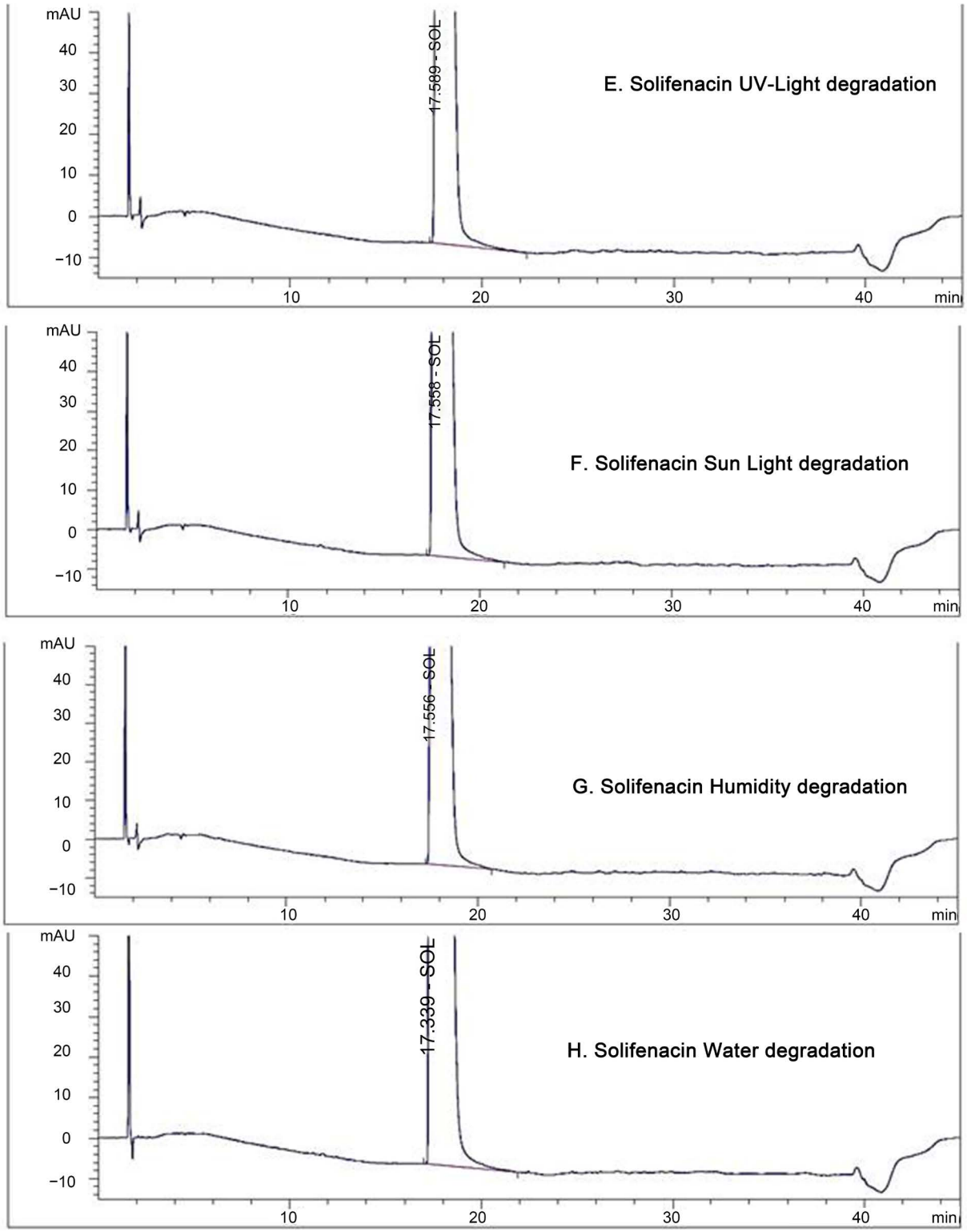
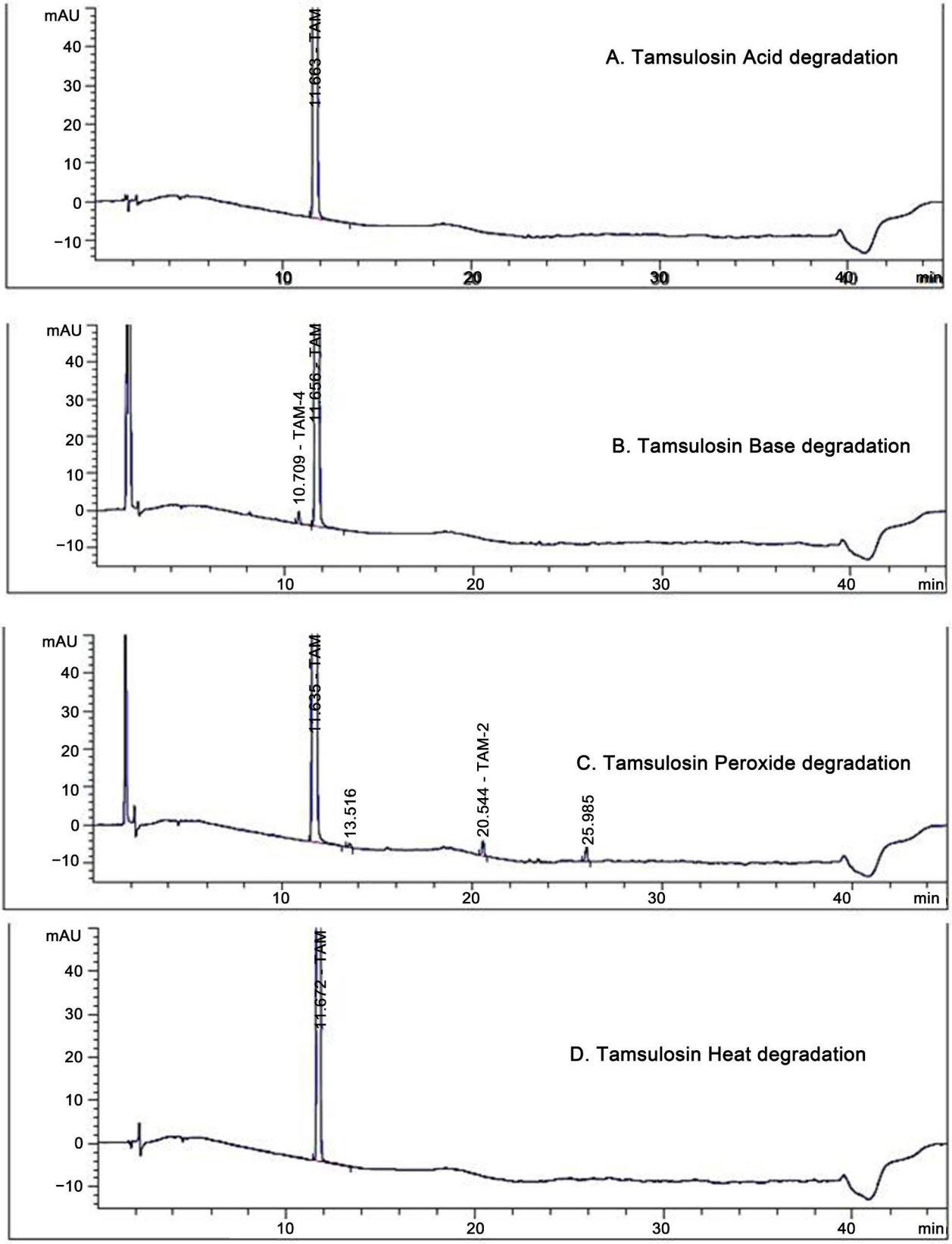


Figure 3. Typical chromatograms of solifenacin at 225 nm (forced degradation study) (A. Solifenacin acid degradation; B. Solifenacin base degradation; C. Solifenacin peroxide degradation and D. Solifenacin heat degradation); Typical chromatograms of solifenacin at 225 nm (forced degradation study) (E. Solifenacin UV-light degradation; F. Solifenacin sunlight degradation; G. Sol ifenacin humidity degradation and H. Solifenacin water degradation).


mAU
40
30
20
10
0
−10
11.663 - TAM
A. Tamsulosin Acid degradation
10
20
30
40
min
mAU
10.709 - TAM-4
11.656 - TAM
B. Tamsulosin Base degradation
mAU
11.635 - TAM
13.516
20.544 - TAM-2
25.985
C. Tamsulosin Peroxide degradation
mAU
11.672 - TAM
D. Tamsulosin Heat degradation

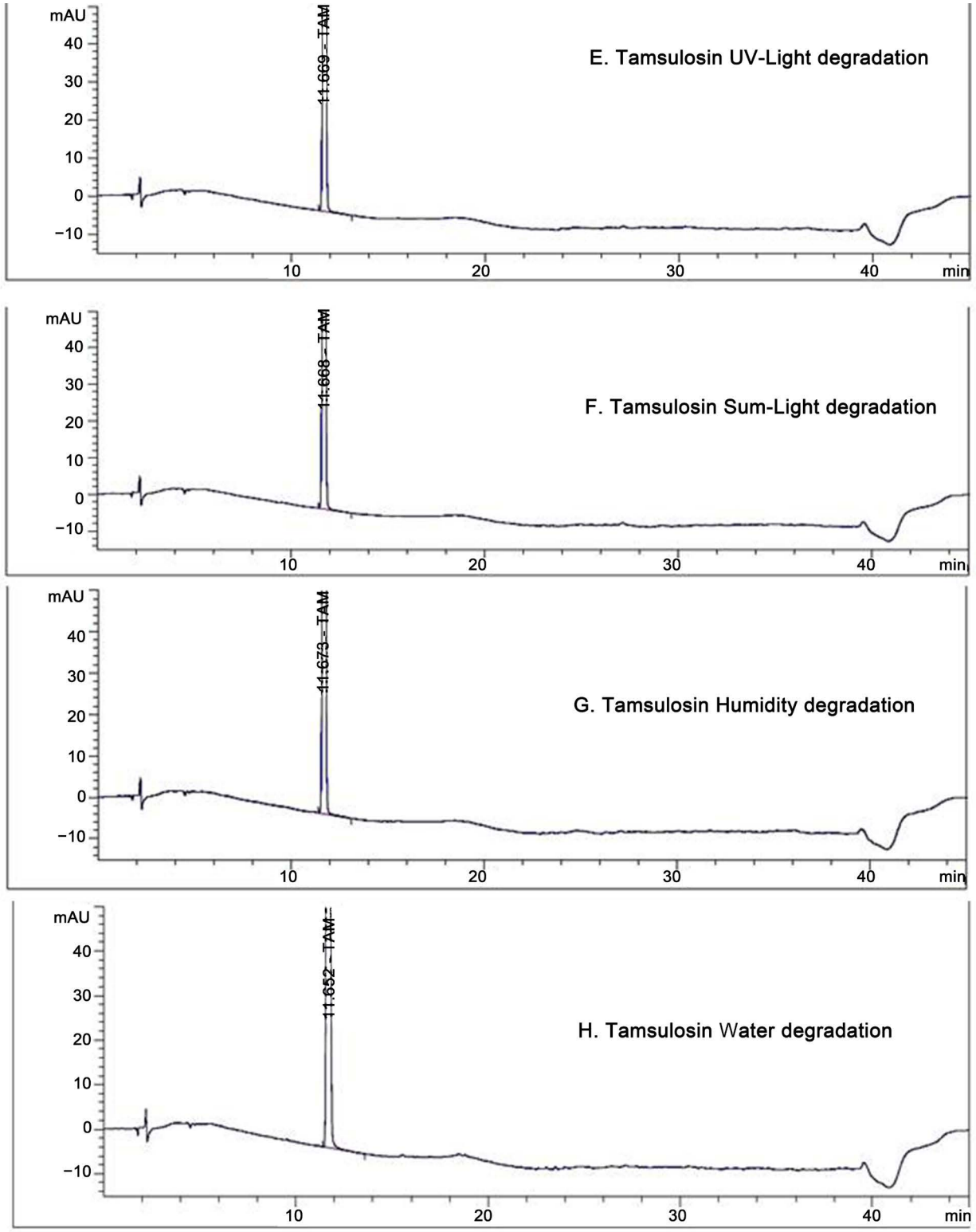


Figure 4. Typical chromatograms of tamsulosin at 225 nm (forced degradation study) (A. Tamsulosin acid degradation; B. Tamsulosin base degradation; C. Tamsulosin peroxide degradation and D. Tamsulosin heat degradation); Typical chromatograms of solifenacin at 225 nm (forced degradation study) (E. Tamsulosin UV-light degradation; F. Tamsulosin sun-light degradation; G. Tamsulosin humidity degradation and H. Tamsulosin water degradation).

Table 2. Specificity.

Stress condition (Degradation)	% of impurities formed (% of area normalization)						Peak purity†	
	SOL-1	SOL-2	TAM-4	TAM-2	Unknown 25.985 RT	% of degradation	TAM	SOL
As such sample	ND*	ND	ND	ND	ND	ND	997.501	997.750
Oxidation	0.1196	ND	ND	0.1583	0.1673	0.491	999.485	999.879
Acid	ND	ND	ND	ND	ND	ND	998.525	999.865
Base	ND	0.0458	0.1771	ND	ND	0.2229	997.858	999.735
Thermal	ND	ND	ND	ND	ND	ND	999.777	999.869
Water	ND	ND	ND	ND	ND	ND	997.713	999.836
UV	ND	ND	ND	ND	ND	ND	999.534	999.899
SUN	ND	ND	ND	ND	ND	ND	999.418	999.907
Humidity	ND	ND	ND	ND	ND	ND	999.445	999.904

*ND-Not detected; †Peak purity numbers represented as per Agilent Chemstation software algorithm. Peak is pure only if purity value is more than 990.

Table 3. Regression and precision data.

PARAMETER	TAM	SOL	TAM-1	TAM-2	TAM-3	TAM-4	SOL-1	SOL-2	SOL-3
LOD (µg/mL)	0.0135	0.0750	0.0153	0.0154	0.0306	0.0198	0.0538	0.0437	0.0451
LOQ (µg/mL)	0.0432	0.2211	0.0466	0.0432	0.0897	0.0610	0.1630	0.1346	0.1362
Correlation coefficient	0.9998	0.9998	1.0000	1.0000	0.9998	1.0000	0.9997	1.0000	1.0000
Bias at 100% response	0.7791	0.5462	0.4888	0.4198	1.0061	0.1943	1.1538	0.3881	0.4176
Precision (%RSD)	1.3	2.0	2.3	3.4	1.5	2.1	2.6	0.6	1.1
Intermediate precision (%RSD)	0.7	1.0	1.6	1.2	1.2	0.8	0.4	2.0	1.6
Precision at LOQ (%RSD)	2.9	3.5	4.2	4.5	2.0	2.8	3.4	2.7	3.3

Table 4. Evaluation of accuracy.

Amount spiked*	% of Recovery†								
	TAM	SOL	TAM-1	TAM-2	TAM-3	TAM-4	SOL-1	SOL-2	SOL-3
LOQ	98.8 ± 2.5	98.7 ± 3.1	98.8± 2.3	99.3 ± 2.2	98.9 ± 0.6	99.6 ± 1.8	100.0 ± 3.3	101.0 ± 2.6	100.9 ± 2.1
50%	99.4 ± 1.0	99.6 ± 1.0	99.1 ± 0.2	99.5 ± 0.5	99.4 ± 1.5	100.1 ± 0.9	101.3 ± 2.1	101.2 ± 0.4	101.0 ±0.5
100%	100.2 ± 0.4	99.4 ± 2.0	100.4 ± 0.7	100.0 ± 1.1	100.3 ± 0.7	100.1 ± 1.3	99.9 ± 0.6	101.3 ± 0.6	100.2 ± 0.7
150%	98.9 ± 2.3	99.0 ± 0.4	100.0 ± 0.9	100.7 ± 0.8	99.7 ± 1.3	101.0 ± 1.4	100.2 ± 0.5	100.6 ± 1.6	101.2 ± 0.8
200%	98.6 ± 2.5	100.6 ± 0.7	99.8 ± 0.3	100.3 ± 0.7	100.6 ± 0.3	99.9 ± 1.3	99.9 ± 0.4	101.8 ± 0.4	100.8 ± 1.1

*Amount of seven impurities spiked with respect to 0.5% specification level individually to Tamsulosin and Solifenacin. †Mean ± %RSD for three determinations at each level.

Table 5. Regression and precision data-assay.

PARAMETER	TAM	SOL
Correlation coefficient	0.9999	1.0000
Bias at 100% response	1.2114	0.5568
Precision (%RSD)	0.3	0.3
Intermediate precision (%RSD)	0.2	0.1

Table 6. Evaluation of accuracy in assay.

Amount spiked*	% of Recovery†	
	TAM	SOL
50%	99.8 ± 0.9	100.1 ± 0.1
75%	100.2 ± 0.5	99.9 ± 0.3
100%	99.9 ± 0.2	99.9 ± 0.2
150%	100.0 ± 0.2	99.9 ± 0.2
200%	100.1 ± 0.3	99.8 ± 0.2

†Mean ± %RSD for three determinations at each level.

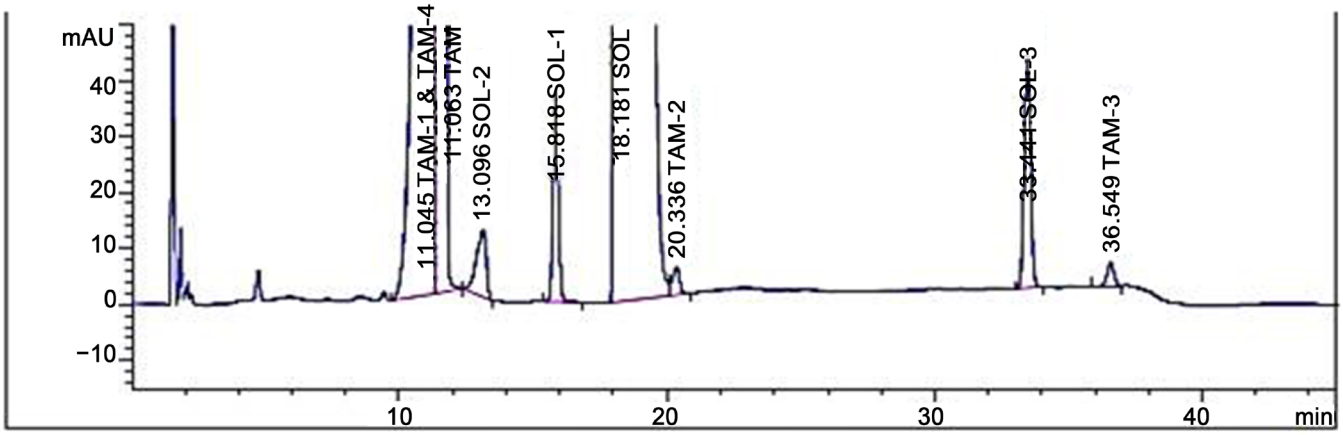
TAM-4 peak shapes not good. And SOL-2 peak not resolved well from TAM peak and TAM-2 peak co-eluted with SOL peak. Further checked with different brand C-18 column Hypersil BDS (C-18, 150-mm 4.6-mm, 5 µm particles) and found resolution less than 2.0 between TAM & SOL-2 peaks and TAM-2 peak co-eluted with SOL due to broad peak shape of SOL, which may be because of SOL interaction with residual silanol groups of stationary phase. Hence experiments were tried using Triethyl amine as mobile phase additive to end cap the silanol groups.

Triethylamine (TEA) is widely used as a mobile phase additive to reduce peak tailing when analyzing bases at neutral pH. TEA acts as competing base, reducing the availability of stationary phase silanols and interaction of the analyte with the silanols.

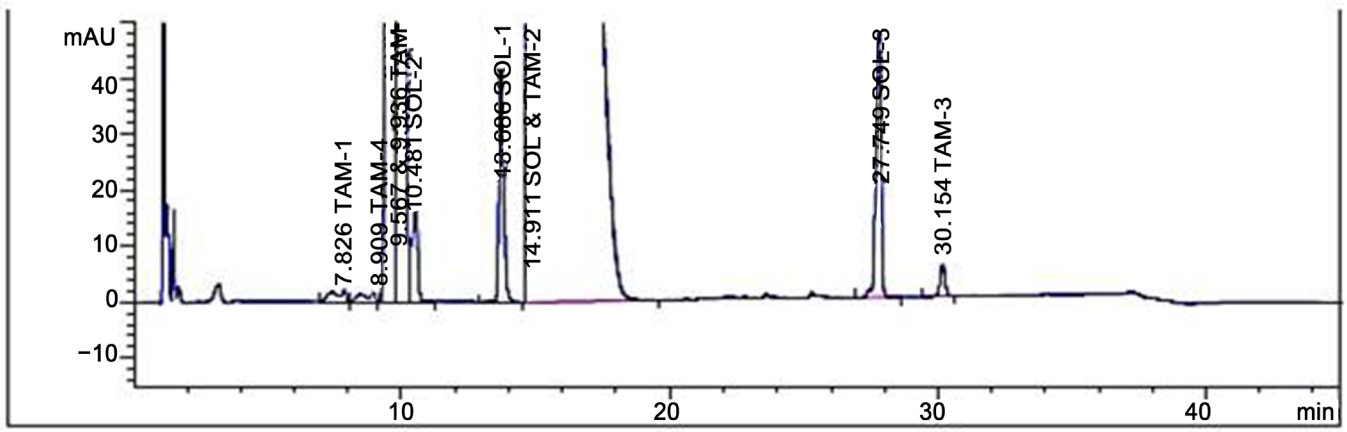
To increase the resolution between SOL-2 & TAM peaks and to resolve TAM-2 peak from SOL peak by reducing SOL peak broadness, introduced 0.5% Triethyl amine as organic modifier in solvent-A (20 mM monobasic potassium phosphate buffer) at two different pH (6.5 & 3.0) conditions respectively, and column used Hypersil BDS (C-18, 150-mm 4.6-mm, 5 µm particles). pH 6.5 buffer results found that resolution increased a little bit between SOL-2 & TAM peaks and SOL peak broadness drastically decreased and TAM-2 peak resolved from SOL peak. pH 3.0 buffer results found that TAM-1 & TAM-4 peak shapes found not symmetric and resolution between SOL-1 & SOL and SOL & TAM-2 found less than 2.0. Among these experiments, buffer solution of mobile phase with 0.5% triethylamine pH adjusted to 6.5 with ortho phosphoric acid had shown better resolution and improved peak shapes, using C18 column. However it is necessary to improve the resolution between TAM & SOL-2; SOL-1 & SOL. Effect of variations was studied with respect to buffer pH, flow rate and column oven temperature. Based on the experiments with different gradient programs a gradient program (T/%B): 0/20, 10/40, 15/40, 22/70, 37/70, 38/20 and 45/20 at detection wavelength 225 nm with the mobile phase consists of solvent-A (20 mM Monobasic potassium phosphate buffer with 0.5% triethylamine, pH adjusted to 6.6 with phosphoric acid) and solvent-B (Milli-q water and acetonitrile in the ratio 10:90 v/v), using Capcell Pak C18, MG, 150 × 4.6 mm; 5 µm column was found suitable for separation of TAM, SOL and its impurities from each other at column oven temperature of 30˚C. The resolution between any two consecutive peaks is more than 2.0 and shapes of all peaks were found symmetric.

Trial chromatograms are shown in Figure 5(A) and Figure 5(B).

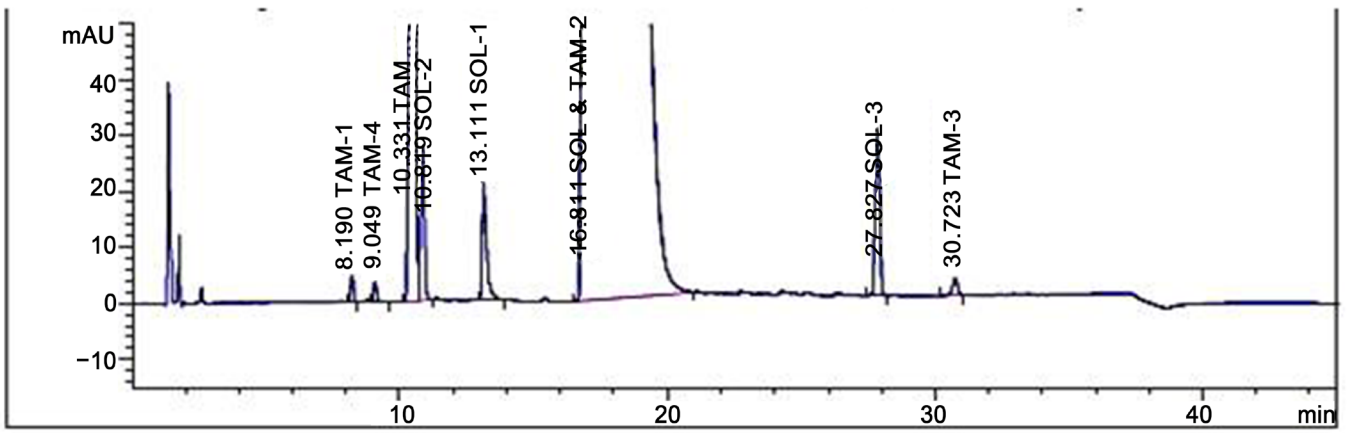
a. Inertsil ODS-3V; 150*4.6mm; 5µm; pH 6.5



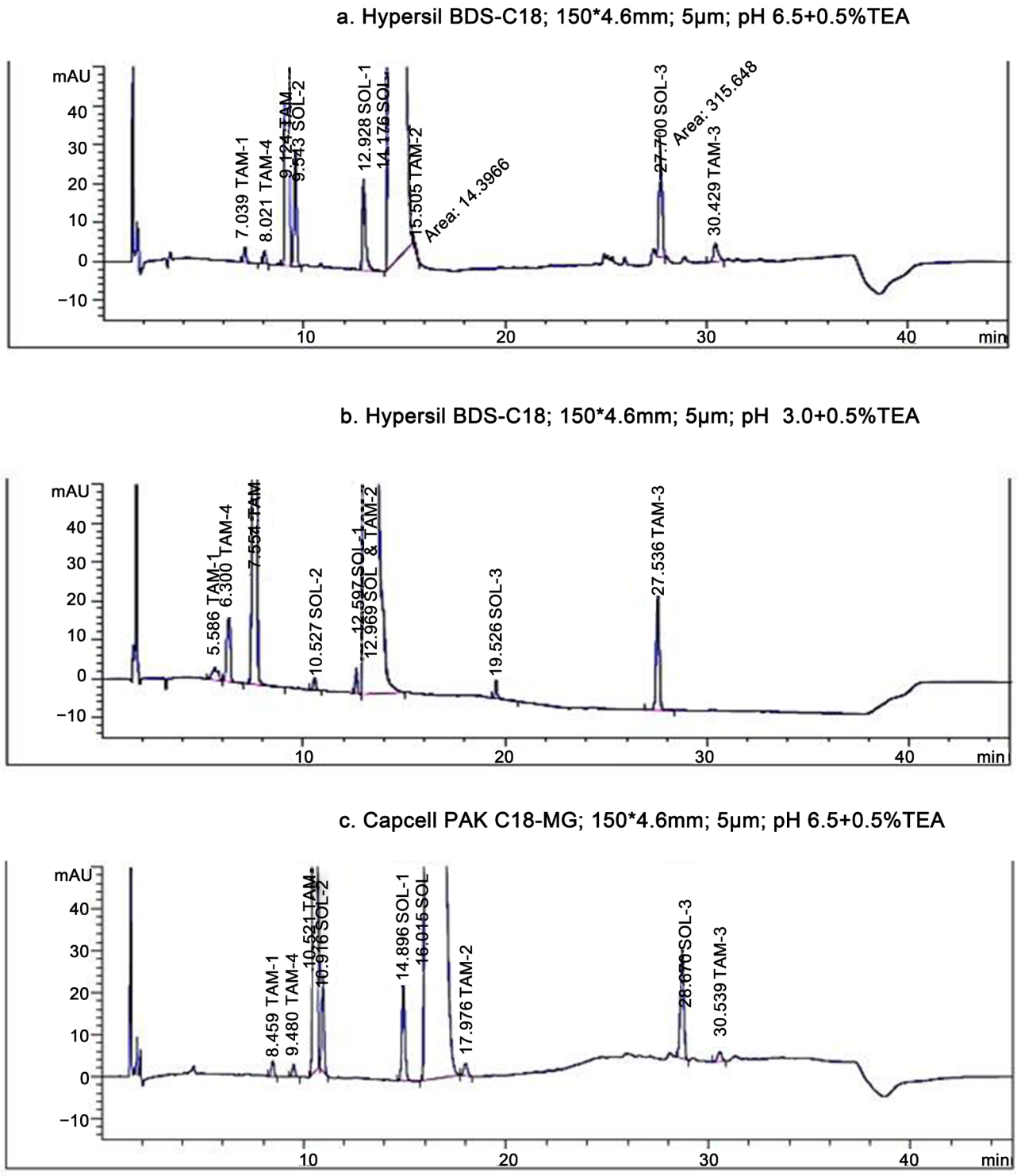
b. Kromasil C8; 150*4.0mm; 5µm; pH 6.5



c. Hypersil BDS-C18; 150*4.6mm; 5µm; pH 6.5



(A)



(B)

Figure 5. (A) Method development trial chromatograms column study Solvent-A pH at 6.5 (a. Inertsil ODS-3V; 150*4.6 mm; 5 µm; b. Kromasil C8; 150*4.0 mm; 5 µm and c. Hypersil BDS-C18; 150*4.6 mm; 5 µm); (B) Method development trial chromatograms Solvent-A pH study at 6.5 & 3.0 with 0.5% TEA (a. Hypersil BDS-C18; 150*4.6 mm; 5 µm; pH 6.5 + 0.5% TEA; b. Hypersil BDS-C18; 150*4.6 mm; 5 µm; pH 3.0 + 0.5% TEA and c. Capcell PAK C18-MG; 150*4.6 mm; 5 µm; pH 6.5 + 0.5% TEA).

5.2. Wavelength Justification

Wavelength was selected based on wavelength maxima of SOL, TAM and their known impurities UV spectrums. SOL and TAM have wavelength maxima at about 225 nm and their known impurities have sufficient absorbance for the measurement. Hence the 225 nm was selected as suitable wavelength for the estimation of impurities. The UV spectrums of SOL, TAM and their impurities has presented in **Figure 6(A)** & **Figure 6(B)**.

5.3. Method Validation

5.3.1. Solution Stability

Assay (%) of both drugs during solution stability experiments were within ±1%. The variability in the estimation of SOL and TAM impurities were within ±10% during solution stability experiment. The results from solution stability experiments confirmed that standard and sample solutions were stable up to 24 hrs for both assay and related substances analysis.

5.3.2. Specificity

To confirm the specificity all the seven impurities were spiked on test solution and injected into the HPLC system with PDA detector. And the results found that no interference observed at SOL & TAM peaks, hence this method is specific to its intended use.

All forced degradation samples were analyzed at an initial concentration 4800 $\mu g \cdot mL^{-1}$ of SOL and 320 $\mu g \cdot mL^{-1}$ of TAM with LC conditions using PDA detector to ensure the homogeneity and purity of SOL and TAM peaks. During the stress studies it was observed SOL and TAM were degraded significantly at particular conditions. SOL-1 impurity observed at oxidative (1% H_2O_2 at 25˚C for 15 hr) condition (**Figure 3(C)**) and SOL-2 & TAM-4 impurities observed at base hydrolysis (1 N NaOH at 60˚C for 18 h) condition (**Figure 3(B)** & **Figure 4(B)**). TAM-2 and one major unknown impurity at 25.985 min were observed at oxidative (1% H_2O_2 at 25˚C for 15 h) condition (**Figure 4(C)**).

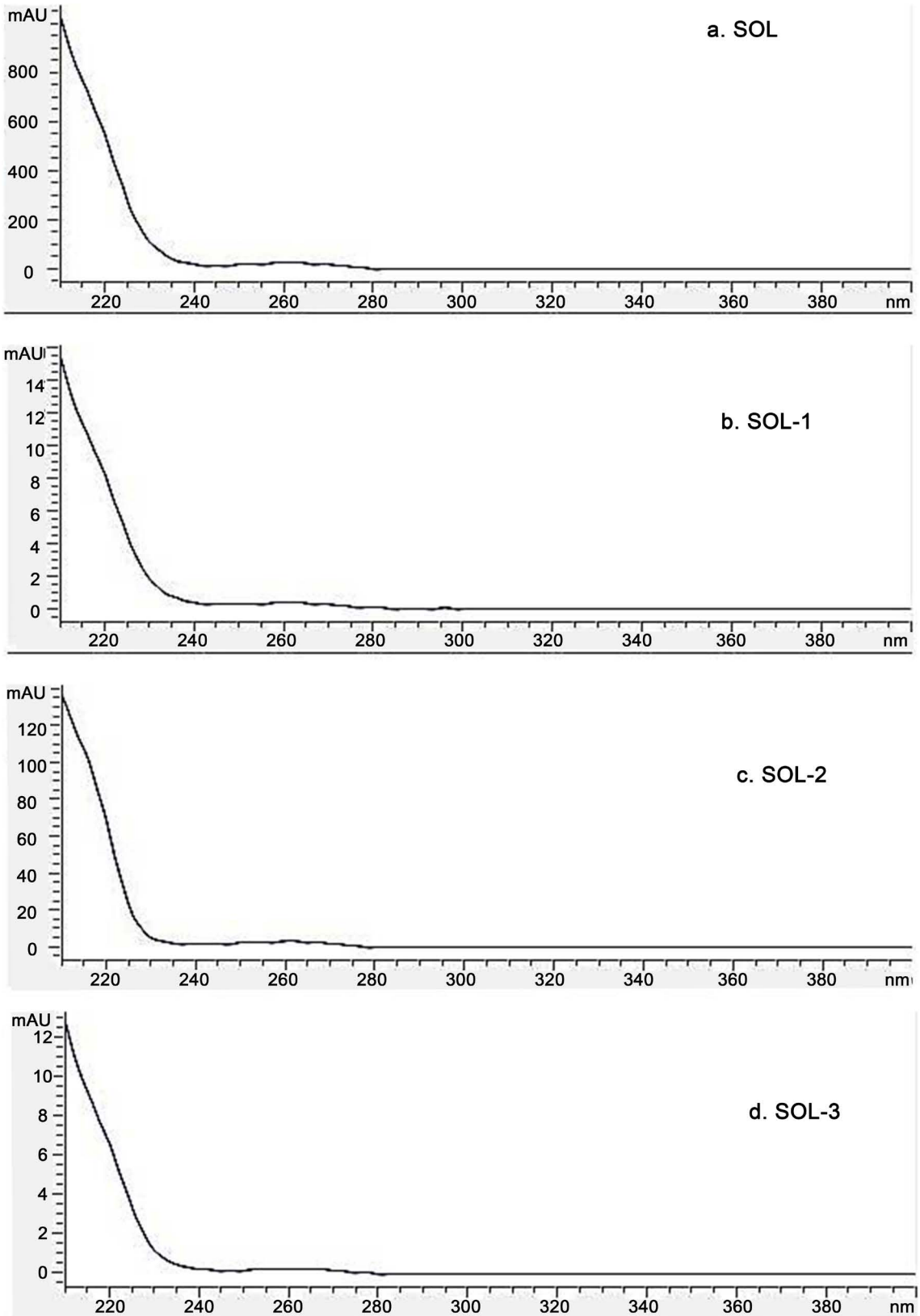
The peak purity test result derived from photo diode array detector (PDA) confirmed that SOL and TAM peaks were pure and homogeneous in all the analyzed stress conditions and thus confirms the stability-indicating power of the developed method. Results of forced degradation studies were reported in **Table 2**.

5.3.3. Linearity

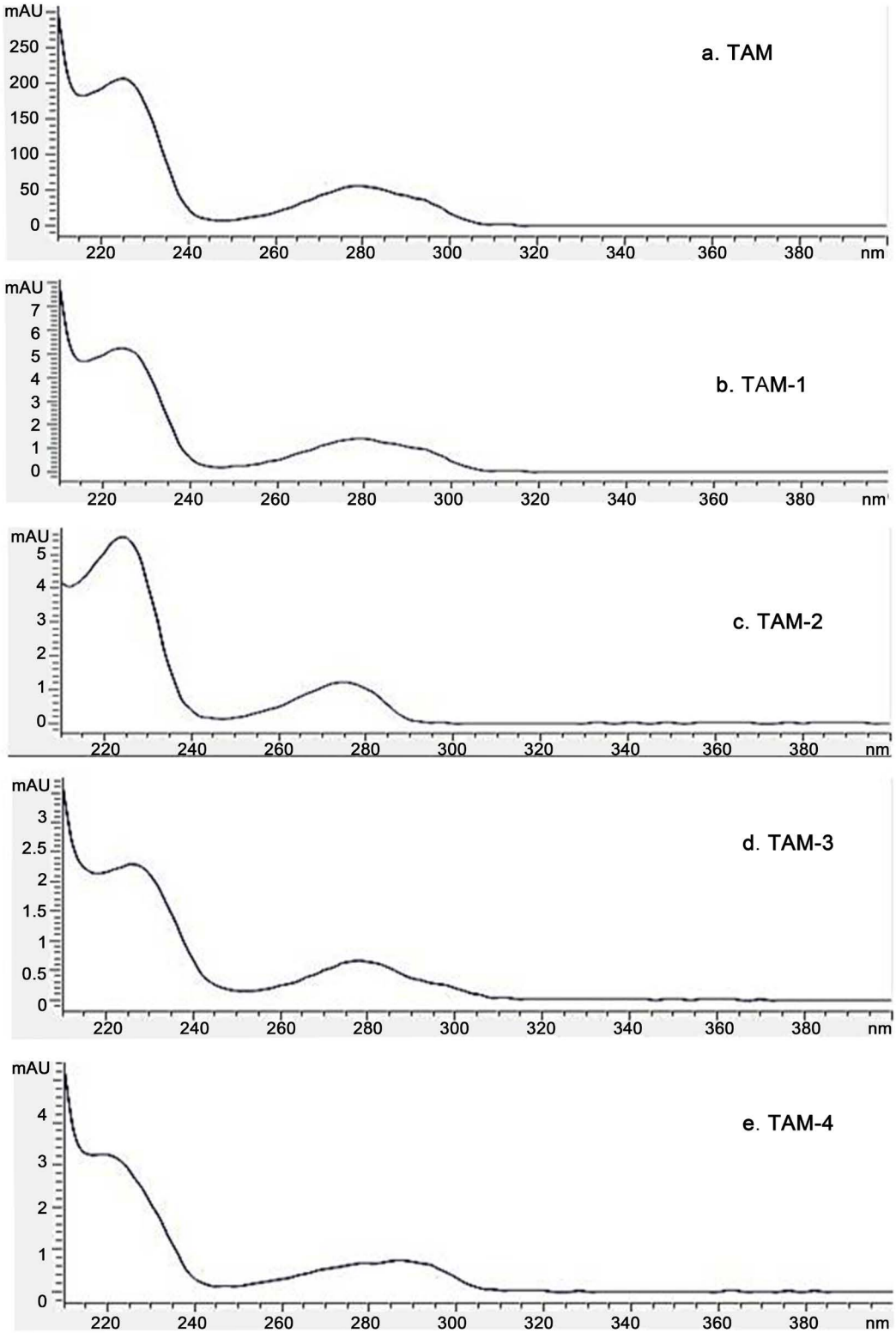
The linearity calibration plot for the assay method was obtained over the calibration ranges tested and correlation coefficient obtained was greater than 0.999 (**Table 5**) for both SOL and TAM. Linear calibration plot for impurities was obtained over the calibration ranges tested, *i.e.* LOQ to 1.0% for impurities. The correlation coefficient obtained was greater than 0.998 (**Table 3**). The above result show that an excellent correlation existed between the peak area and the concentration of all seven impurities.

5.3.4. Limits of Detection and Quantification

The limit of detection and limit of quantification values were established based on the signal to noise ratios. Precision at LOQ values for SOL and TAM and its seven impure-


mAU
800
600
400
200
0
220
240
260
280
300
320
340
360
380
nm
a. SOL
mAU
14
12
10
8
6
4
2
0
b. SOL-1
mAU
120
100
80
60
40
20
c. SOL-2
mAU
12
10
8
6
4
2
d. SOL-3

(A)



(B)

Figure 6. (A) UV spectrum of SOL and their impurities (a. SOL; b. SOL-1; c. SOL-2; d. SOL-3); (B) UV Spectrum of TAM and their impurities (a. TAM; b. TAM -1; c. TAM -2; d. TAM -3; e. TAM-4).

ties were established and reported in Table 3.

5.3.5. Accuracy

The percentage recovery was ranged from 99.8% to 100.1% for SOL and from 99.8% to 100.2% for TAM in the assay analysis and the results are presented in Table 6. The percentage recovery of impurities varied from 98.8% to 101.8% in the analysis of impurities. The % recovery values for SOL and TAM and their impurities are presented in Table 4.

5.3.6. Precision

The assay results of SOL and TAM during the assay method repeatability study was showed, less than 2.0% RSD of SOL and TAM respectively. Similarly the results of all the impurities (SOL-1, SOL-2, SOL-3, TAM-1, TAM-2, TAM-3 and TAM-4) in related substance method repeatability study was showed less than 15% RSD for each individual impurity. Data of repeat experiment was showed less than 0.3% RSD for Assay and less than 4.5% RSD for impurities. These results are conforming good precision of the method. The % RSD values are presented in Table 3.

5.3.7. Robustness

In all the deliberate varied chromatographic conditions like flow rate (−0.2 $mL \cdot min^{-1}$ and +0.2 $mL \cdot min^{-1}$ of 0.9 $mL \cdot min^{-1}$), column temperature (±5˚C of 30˚C), and pH of mobile phase buffer (±0.1 pH 6.6), all analytes were adequately resolved and elution orders remained unchanged. The resolution between all pair compounds was greater than 2.0 and tailing factor for SOL and TAM and their impurities was less than 1.5. The variability in the estimation of SOL and TAM impurities was within ±10%.

6. Conclusion

The rapid reproducible gradient RP-HPLC method developed for quantitative analysis of SOL and TAM and related substances in pharmaceutical dosage form is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the method. The method is stability-indicating and can be used for routine analysis of production samples to check the stability [26] of SOL and TAM in combined dosage form.

References

[1] Astellas Pharma Europe BV (2014) Vesomni 6 mg/0.4 mg Modified-Release Tablets. Public Assessment Report of the Medicines Evaluation Board in the Netherlands, Solifenacin Succinate/Tamsulosin Hydrochloride, Vesomni SPC, The Netherlands. https://www.medicines.org.uk/emc/medicine/28535

[2] Lim, K.T., Kim, Y.T., Lee, T.Y. and Park, S.Y. (2011) Effects of Tamsulosin, Solifenacin, and Combination Therapy for the Treatment of Ureteral Stent Related Discomforts. *Korean Journal of Urology*, **52**, 485-488. http://dx.doi.org/10.4111/kju.2011.52.7.485

[3] Nazir, J., Heemstra, L., van Engen, A., Hakimi, Z. and Ivanescu, C. (2015) Cost-Effectiveness of a Fixed-Dose Combination of Solifenacin and Oral Controlled Adsorption System

Formulation of Tamsulosin in Men with Lower Urinary Tract Symptoms Associated with Benign Prostatic Hyperplasia. *BioMed Central*, **15**, 41.

[4] Solifenacin Succinate API Monograph EP 8.6.

[5] Tamsulosin Hydrochloride API Monograph USP-38 NF33 S2.

[6] Tamsulosin Hydrochloride API Monograph EP 8.0.

[7] Tamsulosin Hydrochloride Capsules Monograph USP-38 NF33 S2.

[8] Yanagihara, T., Aoki, T., Soeishi, Y., Iwatsubo, T. and Kamimura, H. (2007) Determination of Solifenacin Succinate, a Novel Muscarinic Receptor Antagonist, and Its Major Metabolite in Rat Plasma by Semi-Micro High Performance Liquid Chromatography. *Journal of Chromatography B*, **859**, 241-245. http://dx.doi.org/10.1016/j.jchromb.2007.10.005

[9] Rami Reddy, B.V., Srinivasa Reddy, B., Raman, N.V.V.S.S., Subhash Reddy, K. and Rambabu, C.(2012) Development and Validation of a Specific Stability Indicating High Performance Liquid Chromatographic Methods for Related Compounds and Assay of Solifenacin Succinate. *Journal of Chemistry*, **2013**, Article ID: 412353.

[10] Radha Krishna, S., Rao, B.M. and Someswara Rao, N. (2010) A Validated Rapid Stability-Indicating Method for the Determination of Related Substances in Solifenacin Succinate by Ultra-Fast Liquid Chromatography. *Journal of Chromatographic Science*, **48**, 807-810. http://dx.doi.org/10.1093/chromsci/48.10.807

[11] Chandra Mohan, T., Hemalatha, B., Shainy, B., Vasundhara, G., Sandhya, S. and Ashok Kumar, A. (2014) A Rapid RP-HPLC Method Development and Validation for the Quantitative Estimation of Solifenacin Succinate in Tablets. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**, 201-204.

[12] Shaik, R.P., Puttagunta, S.B., Bannoth, K.C. and Reddy Challa, B.S. (2014) Analytical Method Development and Validation of Solifenacin in Pharmaceutical Dosage Forms by RP-HPLC. *ISRN Analytical Chemistry*, **2014**, Article ID: 132020.

[13] Desai, N., Hussen, S.S., Vasanthraju, S.G., Karthik, A. and Udupa, N. (2010) Development & Validation of Stability Indicating HPLC Method for Determination of Solifenacin in Bulk Formulations. *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**, 70-74.

[14] Puttagunta, S.B., Shaik, R.P., Bannoth, C.K., Reddy Challa, B.S. and Awen, B.Z. (2014) Bioanalytical Method for Quantification of Solifenacin in Rat Plasma by LC-MS/MS and Its Application to Pharmacokinetic Study. *Journal of Analytical Science and Technology*, **5**, 35. http://dx.doi.org/10.1186/s40543-014-0035-0

[15] Divya Teja, G., Deva Dasu, C.H., Srinivasa Babu, P. and Ravisankar, P. (2013) Quantitative Analysis of Solifenacin Succinate in Pharmaceutical Dosage Form Using UV Absorption Spectroscopy. *Journal of Chemical and Pharmaceutical Sciences*, **6**, 195-198.

[16] Sudha, T. and Dhomane, J. (2011) A Validated RP-HPLC Method for the Determination of Impurities in Tamsulosin HCL. *International Journal of Chemistry Research*, **2**, 29-33.

[17] Santhi Priya, M., Shanta kumari, K., Bhanu Prasad, M., Priyanka, G. and Sirisha, T. (2014) Impurity Method Development and Validation of Tamsulosin Hydrochloride by Using RP-HPLC. *World Journal of Pharmaceutical Research*, **3**, 998-1005.

[18] Basniwal, P.K., Panda, S., Jain, S. and Deepti, J. (2012) Stability-indicating HPLC Assay Method and Degradation Profile of Tamsulosin. *American-Eurasian Journal of Scientific Research*, **7**, 193-198.

[19] Dunn, C.J., Matheson, A. and Faulds, D.M. (2002) Tamsulosin: A Review of Its Pharmacology and Therapeutic Efficacy in the Management of Lower Urinary Tract Symptoms. *Drugs Aging*, **19**, 135-161. http://dx.doi.org/10.2165/00002512-200219020-00004

[20] Ramakrishna, N.V., Vishwottam, K.N., Manoj, S., Koteshwara, M., Wishu, S. and Varma, D.P. (2005) Rapid, Simple and Highly Sensitive LC-ESI-MS/MS Method for the Quantification of Tamsulosin in Human Plasma. *Biomedical Chromatography*, **19**, 709-719. http://dx.doi.org/10.1002/bmc.498

[21] Vijaya Saradhi, S., Meherjaha, S.K., Prithvi Prasad, Y., Baby Sirisha, P. and Bala Sekaran, C. (2013) RP-HPLC Method for the Estimation of Tamsulosin in Bulk and Capsules. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**, 342-345.

[22] Vijaya Saradhi, S., Meherjaha, S.K., Jyothsna, N., Priyanka, A., Baby Sirisha, P., Ramakrishna, C. and Bala Sekaran, C. (2012) Novel Spectroscopic Methods for the Determination of Tamsulosin in Bulk and Capsules. *Journal of Pharmaceutical Sciences and Research*, **4**, 1958-1963.

[23] Siva Rama Krishna, G.V., Janardhan, M. and Rasool, S.K. (2012) Development and Validation of Stability-Indicating RP-HPLC Method for Estimation of Tamsulosin HCL Pellets. *International Journal of Pharmaceutical Invention*, **2**, 51-60.

[24] Israel, D.S., Krishnachaitanya, K. and Gowri Sankar, D. (2013) RP-HPLC Method for the Estimation of Tamsulosin and Solifenacin in Bulk and Its Dosage Forms. *International Journal of Pharmaceutical Sciences and Research*, **4**, 4343-4350.

[25] International Committee on Harmonization (ICH) (2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1). IFPMA, Geneva.

[26] International Committee on Harmonization (ICH) (2003) Stability Testing of New Drug Substances and Products Q1A (R2). IFPMA, Geneva.