High-Performance Liquid Chromatographic Stability Indicating Assay Method of Tianeptine Sodium with Simultaneous Fluorescence and UV Detection

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Abstract

The purpose of this work is to develop a sensitive, selective, and validated stability-indicating HPLC assay of tianeptine (TIA) in bulk drug and tablet form. TIA is subjected to different stress conditions, including UV-light, oxidation, acid base–base hydrolysis, and temperature. TIA and its possible degradation products are analyzed on Agilent-Zorbax-XDB-C18 column using gradient elution with acetonitrile and 0.02M sodium acetate (pH 4.2). The samples are monitored simultaneously with photo-diode array at 254 nm and fluoroscence detector set to 350 nm (ex) and 425 nm (em). TIA is integrated from its UV-chromatogram, and the photodecomposition products are integrated from the fluoroscence-chromatogram. TIA and its photodecomposition products are separated by TLC using ethyl acetate–*n*-hexane–glacial acetic acid–methanol (10.0:14.0:0.2:1.0, v/v) as developing system. One potential photodegradation product is detected by fluoroscence in TIA-tablet form and separated by TLC. The linear range of TIA is between 0.5 to 50 µg/injection with limits of quantitation and detection values of 30 and 8 ng/injection, respectively. The inter-assay percentage of deviation is not more than 0.03%, and the day-to-day variation is not more than 0.1%.

Introduction

Tianteptine (TIA), 7-{[3-Chloro-6,11-dihydro-6-methyl-dibenzo(c,f)(1,2)thiazepin-11-yl]amino}heptanoic acid S,S-dioxide, is a new antidepressant effective against anxiety accompanying mood disorders. Its antidepressant and anxiolytic properties and its action on somatic complaints claimed to make the drug particularly suitable for the treatment of the entire range of depressive symptomatology (1).

Nicot et al. (2) described an isocratic reversed-phase ion-pair liquid chromatographic method for the determination of TIA and its two main metabolites in plasma, urine, and tissues. TIA was extracted as an ion pair using a heptane–octanol–tetra-heptylammonium bromide mixture (98:2:0.5, v/v/w) as extraction solvent and measured by UV detection at 220 nm. Also, an improved high-performance liquid chromatography (HPLC) method for the quantitative measurement of TIA and its main metabolite in human plasma has been designed (3). Extraction involved ion-paired liquid–liquid extraction and UV detection at 200 nm. However, a new HPLC determination method for TIA in human plasma with fluoroscence detection has been reported (4). The method was based on derivatization of TIA with 4-chloro-7-nitrobenzofurazan in borate buffer (pH 8.5). TIA has been determined in bulk and pharmaceutical dosage form by a photochemically induced fluorimetric detection method (5). Besides, a thin-layer chromatographic (TLC) method has been described for the identification of TIA in urine (6).

No reports described the stability of TIA or its expected potential impurities. For these reasons, it was essential to monitor TIA

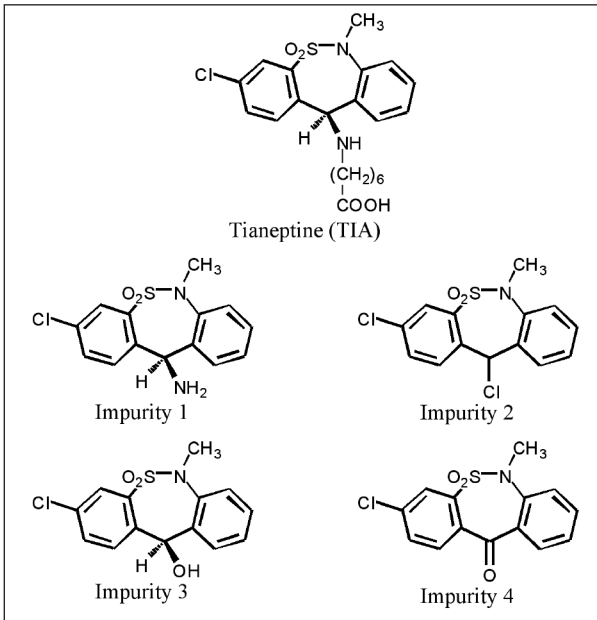

Figure 1. Chemical structure of TIA and its possible impurities.

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degradation products accurately. The US patent 0037021A1 described the method of synthesis of TIA (7). The starting materials and reaction intermediates should be considered for qualification of the bulk powder (Figure 1). In this work, TIA was exposed to different stress conditions and subsequent HPLC analysis with simultaneous detection with UV and fluoroscence detectors.

Experimental

Apparatus

Alliance Waters separations module 2695, Waters 2996 Photodiode array detector set to 254 nm, and Waters 2475 multi lambda fluoroscence detector set to 350 nm as excitation wavelength and 425 nm as emission wavelength were used (Waters, Milford, MA). The column heater was set to 25°C ± 2°C. HPLC system control and data processing were performed by Empower software (Build 1154, Waters). Agilant Zorbax-XDB C18 column (4.6-mm i.d. × 25 cm length, 5-µm particle diameter) protected with an Agilent Zorbax XDB-C18 pre-column (Agilent Technologies, Palo Alto, CA) was used. A ruggedness test was performed using an HPLC µBondapack C_{18} column (3.9-mm i.d. × 300-mm length, 5-µm particle diameter) from Waters (Ireland). A gas chromatograph (GC)–mass spectrometer (MS), Clarus 500 GC/MS (PerkinElmer, Shelton, CT) was used. The software controller/integrator was TurboMass, version 4.5.0.007 (PerkinElmer). An Elite 5MS GC capillary column (30 × 0.25-mm × 0.5 µm, PerkinElmer) was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 2 mL/min (32 p.s.i., flow initial 55.8 cm/s, split; 1:40). Temperature conditions were: inlet line temperature, 200°C; source temperature, 150°C; trap emission, 100°C; and electron energy, 70 eV. The column temperature program was: 50°C for 5 min, increased to 220°C (rate, 20°C/min), and held for 5 min. The injector temperature was 220°C. MS scan was from 50 to 650 *m/z*. Screw-capped borosilicon mini-reaction vials 2-mL (v-shaped, with TFE liners) were used for stress testing (Alltech, GmbH, Unterhaching, Germany). The UV lamp used was 125 cm long (General Electric, Cincinnati, OH). This lamp was installed in a Laminar flow cabinet (Steril Gard Hood, Class II), designed as germicidal UV light source (The Baker Co., Sanford, Maine). TLC silica gel F254 plates (20 × 20 cm) were from E. Merck (Darmstadt, Germany).

Chemicals and materials

All solvents were of HPLC grade (Merck). All other materials were of analytical grade. TIA sodium was obtained as a gift from laboratories of Servier (Gidy, France). Stablon 12.5 mg tablets (contain 12.5 mg TIA sodium salt per tablet) were manufactured by the laboratories of Servier (batch number 4E3251).

Mobile phase

The mobile phase consisted of acetonitrile (solvent A) and 0.02M sodium acetate (solvent B, adjusted to pH 4.2 with 100% acetic acid). The gradient elution of solvent A was from 30% to 65% and solvent B was from 70% to 35%. The starting time was from 0 to 32 min, and the flow rate was 1.0 mL/min.

Solutions

TIA standard solution was prepared by dissolving 12.5 mg ($C_{21}H_{24}ClN_2O_4Na$) in 10 mL methanol and was used as the stock solution for further dilutions. The standard TIA solution was freshly prepared and was not used for more than 24 h, and it was stored away from light. All solvents used as diluents for standard or sample solutions were enriched with dry nitrogen gas before use. Serial standard solutions of TIA consisting of six different concentration levels (0.01 to 1.0 µg/µL) were prepared. For HPLC analysis, a 50-µL sample volume was injected three times. The chromatograms were monitored simultaneously by UV at 254 nm and fluoroscence detection at 350 nm (ex)/425 nm (em). The peak area of the UV-chromatogram was plotted versus the concentration (ng/injection), and the calibration curve was constructed using a least-square regression equation for the calculation of the slope, intercept, and square of correlation coefficient.

Forced degradation

A standard TIA solution (250 ng/µL, 50 µL injection volumes) was injected before and after each sample analysis. Also, blank experiments were carried out. A system suitability injection was developed each working day.

Effect of heat

Two screw-capped reaction vials were used for this experiment. The first vial contained 20 mg of TIA, and the second vial contained 20 mg TIA with 10 µL water as a source of moisture. Both were stored at 90°C for 2 h in a hot-air oven. The contents of the vial were dissolved in methanol and diluted to give a claimed concentration of 250 ng/µL, and a volume of 50 µL was injected for HPLC analysis. The percentage amounts of TIA that remained were calculated referring to standard TIA solution adjusted from the calibration curve.

Effect of acid and alkali hydrolysis

In a 2-mL reaction vial, a volume of 1 mL of methanolic standard TIA solution (0.5 µg/µL) was evaporated to dryness under a stream of nitrogen gas and a volume of 0.5 mL 1M hydrochloric acid was added. This vial was half-inserted into a block heater at 90°C for 10 min and cooled. A volume of 0.5 mL of 1M NaOH was added for neutralization. A volume of 50 µL was injected for HPLC analysis. The same procedure was repeated, but a 0.5 mL 1M NaOH was used instead of HCl and neutralization with 0.5 mL of 1M HCl.

Effect of oxidation

In a 2-mL reaction vial, a volume of 1 mL of methanolic standard TIA solution (0.5 µg/µL) was evaporated to dryness under a stream of nitrogen gas, and a volume of 0.2 mL 100% hydrogen peroxide and 0.8 mL water were added. This vial was half-inserted into a block heater at 90°C for 10 min. The reaction mixture was cooled, and a volume of 50 µL was injected for HPLC analysis.

Effect of UV light on powder form

A sample film of TIA sodium powder of approximately 0.1-mm thickness in a flat glass dish was subjected to UV light (254 nm) (General Electric, Cincinnati, OH) at a distance of about 1-cm

from the lamp for 3, 6, 12, and 24 h. A claimed concentration of 250 ng/µL of TIA was prepared in methanol (after each exposure time), and 50 µL was analyzed by HPLC. A control TIA sample, protected from light by wrapping in aluminium foil, was analyzed.

At the same time, 10 tablets of Stablon were powdered and exposed to UV irradiation. A claimed concentration of 250 ng/µL of TIA was prepared in methanol (after each exposure time), filtered with a Nylon syringe filtration disks, and 50 µL was analyzed by HPLC.

TLC of photodecomposed TIA

A portion of the diluted and concentrated UV-degraded authentic TIA and Stablon methanolic extract were evaporated to dryness, reconstituted in a minimal methanol, and spotted onto TLC silica-gel F_{254} plates (20 × 20 cm). Also, on the same TLC plate, approximately 5 µL of standard TIA (1 mg/mL) was spotted. The TLC plate was developed in ethyl acetate–*n*-hexane–glacial acetic acid–methanol (10.0:14.0:0.2:1.0, v/v). The migration distance of the developing system was not less than 15 cm. The spots were located by viewing under UV light at 254 and 365 nm. The separated bands were scratched, extracted in methanol, and analyzed by HPLC using the developed method.

GC–MS

Into a 1.5-mL autosampler vial a volume of 1-mL methanolic solution of TIA (50 ng/µL) was dried under nitrogen gas and reconstituted in dichloromethane. Fifty microliters of *N*-methyl-*N*-timethylsilyltrifluoroacetamide (MSTFA) and 50 µL CH_2Cl_2 were added. The sample mixture was heated at 60°C for 5 min, cooled, and 1 µL was injected for GC–MS analysis.

Results and Discussion

The development of a stability-indicating assay method for TIA was necessary because there is no report about the possible degradation products that might arise from exposure to different environmental changes. Through this work, not all degradation products of TIA were detectable by UV at 254 nm, and they showed considerably different responses than TIA itself. This has been proven by simultaneous analysis using fluoroscence and UV detection. The maximum excitation and emission fluoroscence wavelengths were determined by online fluoroscence scanning of the TIA and major decomposition products. The degraded samples of TIA were injected several times by applying several interchangeable chromatographic conditions until the best resolution was achieved. Finally, to obtain the best overall chromatographic conditions, the mobile phase was optimized by examining the effect of pH and the content of acetonitrile or methanol. Besides, other chromatographic variables were investigated, including column temperature, column type, mobile phase flow rate, and the setting of the gradient elution program. The opti mal chromatographic conditions were achieved as described in the Experimental section. Not only were the chromatographic parameters considered, but also the fluoroscence response of the decomposition products was considered. In all cases, the amount of TIA was calculated from a UV-chromatograms.

Selectivity, precision, and performance parameters

The samples of TIA were forcedly degraded by exposure to different stress conditions and were injected six repetitive times for HPLC analysis. The eluted analytes were first passed through a UV detector adjusted at 254 nm, and then through the fluoroscence detector adjusted at 350 (ex) and 425 nm (em). The detectors sequence is an important factor because the back pressure of the UV flow cell was slightly greater than the fluoroscence flow cell. All peaks corresponding to degradation products showed a complete separation from each other and from the major peak of TIA eluted at approximately 12.4 min (Figure 2). The photodecomposition products of TIA were monitored by fluoroscence because they showed a very low UV response; however, the peak corresponding to TIA was monitored by UV detector because it showed a very low fluoroscence response. The system was suitable and precise, as shown in Tables I–III.

Linearity and range

A linear UV response of peak areas for TIA was observed over the range of 0.5 to 50 µg/injection. The squared regression coefficient was 0.9999. The response factor of TIA was 229988 peak area unit per each one microgram injected. The lower limit of quantitation (LOQ) was estimated by satisfying two criteria: the signal-to-noise ratio is not less than 10 and the percent relative standard deviation (%RSD) of five replicate injections of the LOQ solution is less than 6%. LOQ of TIA was 30 ± 0.11 ng/injection, and the limit of detection value was 8 ± 0.51 ng/injection. The amount of each degradation product was calculated based on the percentage loss of the principle amount

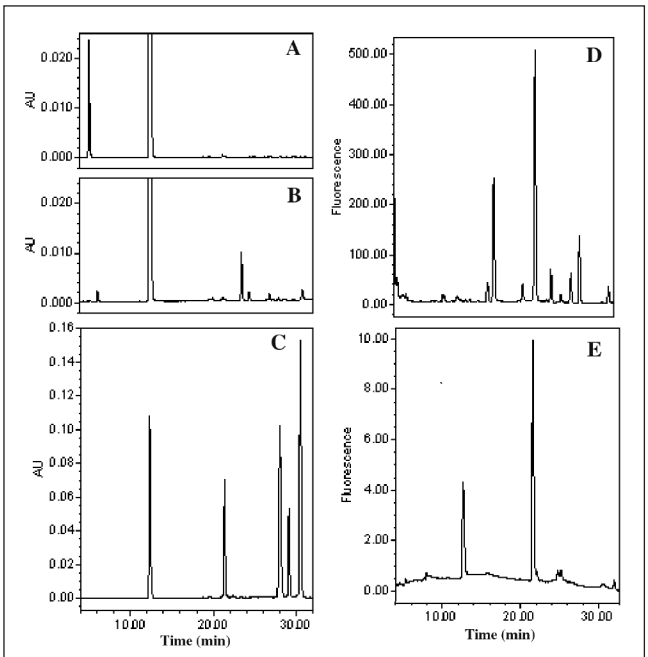

Figure 2. HPLC-chromatograms of TIA (250 ng/injection), heated (A), boiled with NaOH (B), boiled with HCl (C), exposed to UV-254 nm (D), and Stablon tablets extract (E). Chromatograms A, B, and C were monitored by UV at 254 nm, and chromatograms D and E were monitored by fluoroscence detector.

claimed of TIA calculated from its calibration curve. The amount of each decomposition product was calculated from relative percentages of decomposition products to each other. The total amount of degradation products was considered equal to the amount of TIA lost. This method of calculation was followed as per ICH guidelines (8–10).

Table I. Chromatographic Parameters of TIA Degraded with UV light, [Detection; Fluoroscence at 350 nm (ex)/425 nm (em), Claimed TIA Concentration is 25 µg/injection] (*n* = 3)*

t_R (min) (RSD)	Area (RSD)	Width (min)	% Amount ± SD	K'	R (RSD)	α	AS	N
12.39† (0.01)	4312275 (0.04)	0.60	75.00 ± 0.10	5.19			1.10	13644
15.75 (0.21)	948785 (1.22)	0.80	1.72 ± 0.02	6.88	4.80 (0.20)	1.32	0.92	26057
16.80 (0.02)	1438170 (1.22)	1.12	2.60 ± 0.01	7.40	1.09 (0.01)	1.08	1.11	38998
21.81 (0.24)	9831909 (1.06)	1.33	17.80 ± 0.12	9.91	4.09 (0.25)	1.34	1.02	48154
23.96 (0.08)	465015 (0.88)	0.67	0.84 ± 0.21	10.98	2.15 (0.06)	1.11	1.16	106720
27.43 (0.05)	1125420 (0.78)	0.73	2.04 ± 0.05	12.71	4.95 (0.07)	1.16	1.12	80046

* Capacity factor (k′); selectivity coefficient (α); resolution (R); peak asymmetry (AS); and USP plate count (N).
† TIA detected by UV at 254 nm.

Table II. Chromatographic Parameters of TIA Degraded with 1M HCl, (Detection; UV at 254 nm, Claimed TIA Concentration is 250 ng/µL) (*n* = 3)*

t_R (min) (RSD)	Area (RSD)	Width (min)	% Amount ± SD	K′	R (RSD)	α	AS	N
12.31 (0.14)	1397070 (0.06)	0.83	48.60 ± 0.01	5.15			1.11	20393
21.37 (0.02)	694064 (0.88)	0.70	7.32 ± 1.02	9.68	11.82 (0.02)	1.88	1.32	107053
28.05 (0.27)	1759762 (0.75)	1.07	18.55 ± 0.68	13.03	7.57 (0.26)	1.35	1.29	59909
29.17 (0.15)	601828 (0.58)	0.85	6.34 ± 1.33	13.59	1.17 (0.12)	1.04	1.19	152496
30.54 (0.11)	1794384 (0.55)	0.73	18.92 ± 1.20	14.27	1.73 (0.08)	1.05	1.20	157276
32.57 (1.20)	18284 (1.00)	0.47	0.19 ± 0.66	15.28	3.37 (0.95)	1.07	1.13	189448
33.49 (0.02)	7442 (0.65)	0.38	0.08 ± 0.34	15.74	2.17 (0.01)	1.03	1.03	191957

* Capacity factor (k′); selectivity coefficient (α); resolution (R); peak asymmetry (AS); and USP plate count (N).

Accuracy

Analyzing placebo solutions spiked with known amounts of TIA showed the accuracy of the method (claimed, 10, 100, 250, 500, and 1000 ng/injection; found, 10 ± 0.03, 100 ± 0.02, 250 ± 0.05, 500 ± 0.05, and 1000 ± 0.10 ng/injection, respectively). Because the results obtained were within the acceptable range of 100% ± 5%, the method was deemed to be accurate. However, the method accuracy for the determination of potential decomposition products was carried out by repetitive injection of UV degraded TIA sample solution (TIA powder exposed to UV light for 1, 2, and 3 h) of claimed concentration of 500 ng/µL. The amounts of decomposition products were calculated based on the calculated amount of TIA lost.

Robustness

For the evaluation of the method robustness, one chromatographic parameter was changed while the other parameters were kept unchanged. A standard solution (claimed, 500 ng/µL) of photodecomposed TIA powder exposed to UV 254 nm light for 3 h was injected three times after each change. The chromatographic parameters [including capacity factor (k'), retention time (t_R), peak asymmetry (As), resolution (R), and USP width) were calculated and compared with those of the system suitability (Table I). The method robustness was tested after changing the pH of the acetate solution (3.8–4.5), column temperature (25–50°C), and upon using non-end caped C18 HPLC column. The results revealed that the method is robust for these small changes in pH. However, at pH > 4.6, the fluoroscence response of decomposition products were markedly decreased. The method was robust upon using an end caped C18 HPLC column from Agilent; however, the non-end caped column (from Waters) has shown an acceptable resolution with longer retention time (t_R) for all separated peaks. The effect of using 0.1% counter ion in acetate solution was investigated (tetrabutylammonium bromide and *n*-hexanesulfonic acid sodium salt). Both counter ions have shown a dramatic shift of TIA, degradation products, tailed TIA, and ignored fluoroscence response to the decomposition products (Table IV).

Ruggedness

The ruggedness of the method was evaluated by applying the HPLC procedure using two different analysts with two different HPLC systems (Waters Alliance system and 1100 Hewlett Packard HPLC system). Both HPLC systems were equipped with diode array detection and fluoroscence detectors. The %RSD of t_R, k', As, and peak areas obtained with the two chromato-

Table III. Chromatographic Parameters* of TIA Degraded with 1M NaOH, (Detection; UV at 254 nm, Claimed TIA Concentration is 250 ng/µL) (*n* = 3)

t_R (min) (RSD)	Area (RSD)	Width (min)	% Amount ± SD	K'	R (RSD)	α	As	N
6.14 (0.14)	17535 (1.22)	0.33	0.63 ± 1.20	2.07			1.42	11820
12.34 (0.04)	3006823 (0.68)	0.95	95.00 ± 1.10	5.17	9.66 (0.01)	2.50	1.21	20390
23.44 (0.25)	108459 (2.01)	0.70	2.87 ± 0.66	10.72	13.46 (0.21)	2.07	1.21	105459
24.34 (0.10)	21254 (2.03)	0.50	0.53 ± 0.67	11.17	1.50 (0.08)	1.04	1.17	93875
26.76 (0.80)	14480 (2.05)	0.45	0.37 ± 0.55	12.38	5.10 (0.59)	1.11	1.19	122167
30.71 (0.52)	28241 (0.22)	0.60	0.58 ± 0.24	14.36	7.53 (0.52)	1.16	1.13	109795

* Capacity factor (k'); selectivity coefficient (α); resolution (R); peak asymmetry (AS); and USP plate count (N).

Table IV. Relative Standard Deviation of the Chromatographic Parameters of TIA and Its Photodecomposition Products Analyzed with Two Different Columns and Detection with UV for TIA and Fluoroscence for Its Decomposition Products

t_R (RSD)	Area (RSD)	Height	Width	k'	As (RSD)	N (RSD)
Zorbax-XDB						
11.68 (0.01)	899850 (1.55)	81279	0.75	4.84	1.05 (0.00)	24817 (1.22)
15.63 (0.01)	219288 (1.25)	18274	0.70	6.81	1.00 (0.01)	51048 (0.73)
16.32 (0.03)	290420 (2.11)	22198	0.92	7.16	0.98 (0.00)	32452 (1.01)
21.59 (0.01)	2000465 (2.02)	156508	0.97	9.80	1.02 (0.02)	51097 (0.71)
26.23 (0.21)	107390 (1.60)	6822	1.00	12.12	1.20 (0.05)	70455 (0.74)
27.17 (0.11)	252705 (2.66)	18830	0.70	12.58	1.02 (0.01)	154247 (2.17)
µBondapak C_{18}						
15.27 (0.21)	891285 (1.23)	50981	1.15	6.63	1.10 (0.02)	18043 (1.34)
18.29 (0.02)	196955 (1.04)	14532	0.72	8.15	1.03 (0.02)	66709 (0.97)
20.05 (0.04)	269251 (1.44)	13292	1.05	9.02	1.00 (0.00)	37326 (1.55)
23.86 (0.00)	2084414 (2.01)	136871	1.03	10.93	1.01 (0.04)	54596 (1.69)
28.53 (0.01)	115341 (1.24)	6882	0.80	13.26	1.01 (0.01)	130190 (0.69)
30.06 (0.05)	186370 (2.24)	12650	0.68	14.03	1.03 (0.00)	198216 (2.51)

graphic systems and operators were not more than 0.20% and 0.4% for UV and fluoroscence detection, respectively.

Sample solution stability

Sample solution stability was tested by a repetitive daily injection of TIA calibration solution (250 ng/µL) and photodecomposed TIA (250 ng/µL) for four days. The recovered amounts of both solutions were approximately 100% ± 1.1% and 75.0% ± 1.2%, respectively.

Forced degradation of TIA

Approximately 0.03% of the loss was due to boiling at 90°C for 2 h and showed a small peak at 5.2 min in the UV-chromatogram; however, the fluoroscence-chromatogram was indifferent from that of the calibration solution.

The mean TIA is relatively stable at elevated temperature. Also, boiling in alkaline medium has one major degradation peak at 23.4 min (2.9%) with multi-degradation products in the UV-chromatogram and nothing observed in fluoroscence-chromatogram (Figure 2B and Table III). However, boiling in acidic medium has shown relatively high percentages of multi-degradation products eluted at t_R > 21 min, which were detected only by UV-254 nm, and the remaining amount of TIA was approximately 48.6%. None of the TIA acid hydrolysis products were matched with photodecomposition products. The fluoroscence chromatogram of alkali and acid treated TIA samples were clean (Figure 2C and Table II).

Photodecomposition of TIA

The stress testing revealed that TIA is more sensitive toward UV light than other environmental factors.

Intact TIA was detected only in the UV-chromatogram (at 12.4 min) with negligible signals observed in the fluoroscence-chromatogram. However, the UV degradation products were detected much better in fluoroscence-chromatogram compared with negligible or very small signals in the UV-chromatogram.

Approximately 25% of the TIA content was lost after exposure of TIA powder to UV irradiation for 3 h. The color of the powder TIA changed from white to brownish-yellow. The major photodecomposition product was eluted at 21.8 min, with other potential decomposition products eluted at 16.8, 24.0, and 27.4 min (Figure 2D, Table I). The concentrated solution prepared from photodegraded TIA powder sample was also tested by TLC. The developing system was very critical in its composition, and very nice round bands were obtained upon development in

ethyl acetate–*n*-hexane–glacial acetic acid–methanol (10.0: 14.0:0.2:1.0, v/v). The R_f values and retention time (HPLC) were labeled as shown in Figure 3.

GC–MS analysis of pure and UV degraded TIA

TIA was detected only after silylation with MSTFA in CH_2Cl_2 as two peaks at 8.74 and 8.81 min (Figure 4). The most prominent peaks were recorded at *m/z* 217 (50%), which was assigned to the silylated amino acid side chain [NH_2-$(CH_2)_6$-CO-O-Si$(Me)_3$], and *m/z* 117 (10%), which assigned to CO-O-Si$(Me)_3$ fragment.

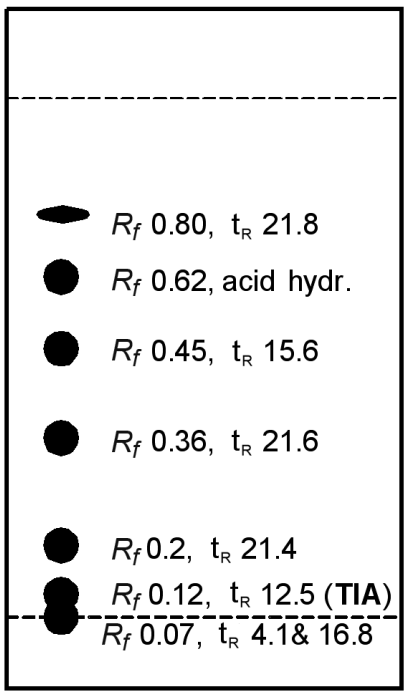

Figure 3. Diagram of TLC of photodegraded TIA, labeled with R_f and retention time of HPLC analysis.

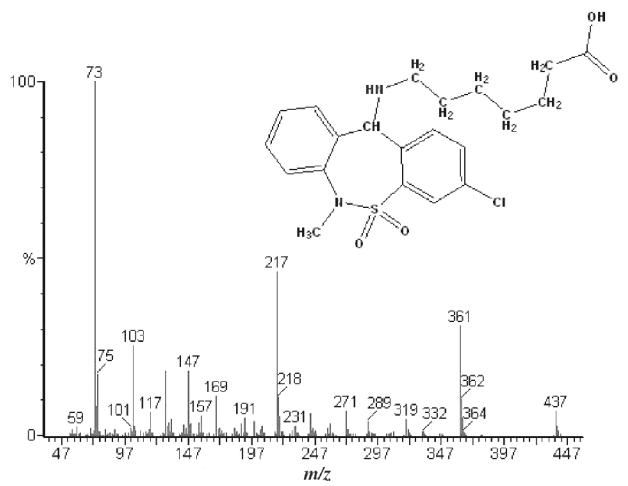

Figure 4. The GC–MS spectrum of TIA derivatized with MSTFA.

Purity testing of Stablon tablets (12.5 mg)

The chromatogram of methanolic solution of Stablon tablet extract (250 ng/µL) showed one impurity (0.1%), which corresponds to peak labeled eluted at 21.4 min (Figure 2E), which was detected only by fluoroscence detection. This peak is claimed to be a result of the effect of light on Stablon tablets. The recovered amount of averaged 10 tablets from each strength were about 101.2% ± 0.43%.

Conclusion

The developed method is able to discriminate between TIA and its possible degradation products. In addition, it has the sensitivity to detect photodecomposition products that could not be detected with UV detector.

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