

# Validated stability indicating HPTLC method for the determination of Tamsulosin hydrochloride in pharmaceutical dosage forms

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**Abstract:** This research paper describes simple, sensitive, precise, specific and stability indicating high-performance thin layer chromatographic method for the determination of Tamsulosin hydrochloride (TAM) in bulk and tablet formulation. Validation was carried out in compliance with International Conference on Harmonization guidelines. The method employed thin-layer chromatography aluminium plates pre-coated with silica gel 60F-254 as stationary phase. The solvent system consisted of toluene-methanol-triethylamine 9:3:1 (v/v/v). This solvent system was found to give compact spots for Tamsulosin hydrochloride with  $R_f$  value  $0.71 \pm 0.02$ . Densitometric analysis of TAM was carried out in the absorbance mode at 282 nm. Linear regression analysis showed good linearity ( $r^2 = 0.9973$ ) with respect to peak area in the concentration range of 100–2000 ng per spot. The method was validated for precision, accuracy and robustness. Pure drug was subjected to acid and alkali hydrolysis, oxidation, photo degradation, dry heat and wet heat treatment. The drug underwent degradation under acidic, basic, oxidative and wet heat conditions. The degraded products were well separated from the pure drug. Statistical analysis proved that the method is reproducible and selective for estimation of TAM in bulk and tablets. As the method could effectively separate the drugs from their degradation products, it can be employed as a stability indicating method.

**Keywords:** Tamsulosin hydrochloride, HPTLC, Stability indicating.

## INRODUCTION

Tamsulosin hydrochloride (TAM), (-) - (R) - 5 - [ 2 - [ [ 2 - ( o - Ethoxy phenoxy) ethyl] amino ] propyl ] - 2 - methoxy benzene sulfonamide monohydrochloride. The chemical structure is shown in Fig. 1 <sup>[1]</sup>. It is selective antagonist of  $\alpha_1$  adrenoceptor located in human prostate and used for the treatment of patients with symptomatic benign prostatic hyperplasia <sup>[2-3]</sup>. Literature survey revealed different analytical methods for determination of TAM. This includes LC-MS for determination of TAM in dog plasma <sup>[4, 5]</sup>, HPLC <sup>[6]</sup>, LC-MS-ESI in human aqueous humor and plasma <sup>[7, 8]</sup>. TAM in human plasma is also determined by HPLC using extraction with butyl acetate and highly sensitive LC-ESI-MS/MS method <sup>[9, 10]</sup>. Voltammetric investigation of Tamsulosin is also found in literature

<sup>[11]</sup> Capillary electrophoretic method is found for chiral separation of TAM <sup>[12]</sup>.

Recently, liquid chromatography coupled to mass spectrometry (LC- MS-MS) has emerged as a powerful tool for the simultaneous estimation of TAM and DUTA in plasma because of its superior sensitivity and selectivity <sup>[13]</sup>. So it was thought of interest to develop stability indicating HPTLC method for the quantitative analysis of TAM and its stress degradation products. The aim of the present work was to develop an economic, accurate, specific, reproducible and stability-indicating HPTLC method using densitometric detection for the determination of TAM in the presence of its degradation products, either in bulk or in tablets.

## EXPERIMENTAL

### Instruments and Apparatus

A camag HPTLC instrument consisted of Linomat V autosprayer, Scanner-III, flat bottom and twin trough developing chambers and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland) were used. HPTLC plates used were of silica gel 60F-254, layer thickness 0.2 mm, 20X10 cm, aluminium backing (E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai). Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

### Reagents and materials

Tamsulosin hydrochloride pure powder with 99.97 % purity was kindly gifted by Intaas pharmaceutical Ltd. All the reagents used during the study were procured from S. d. fine Chemical and were of analytical grade. Tablets were purchased from the local pharmacy.

### Chromatographic Conditions

Before analysis plates of silica gel 60F-254 HPTLC were cleaned by pre-development with methanol and activated at 110°C for 5 min for solvent removal. Solutions of TAM were applied to plates (10X10 cm) by means of a Linomat V automatic spotter equipped with a 100 µl syringe and operated with settings of band length, 6mm; distance between bands, 10 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin trough chamber previously saturated for 30 min with the mobile phase, toluene-methanol-triethylamine 9:3:1 (v/v/v) to 8.5 cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 282 nm for all measurements and operated by the CATS software.

### Preparation of Standard Solution

Accurately weighed 10 mg of TAM was transferred to a 10 ml volumetric flask and dissolved in and diluted up to the mark with methanol to obtain a standard solution of TAM (1000 µg/ml). From this solution, 2 ml was further diluted to 10 ml with methanol to obtain a working standard solution of TAM (200 ng/µl).

### Method Validation

The HPTLC method was validated as per ICH guidelines<sup>[14]</sup>.

### Linearity

Accurate quantities from working standard solutions (1, 2, 4, 6, 8, 10 and 15 µl) were applied to the TLC plate to give bands containing 200–3000 ng of TAM per spot. Each amount was applied five times and the plate was developed, using the previously described optimized mobile phase, and scanned. The calibration curves were constructed by plotting peak areas versus concentrations.

### Accuracy (% Recovery)

Accuracy of the method was determined by standard addition method in which the known amount of standard TAM solutions were added to preanalyzed tablet solution. These amounts corresponded to 50, 100 and 150 % of the amounts claimed on the label. The amounts of TAM were estimated by applying these values to the regression equation of the calibration curve.

### Method Precision (Repeatability)

The precision of the instruments was checked by repeated spotting of same solution and repeated scanning of the same spot ( $n=6$ ) of TAM without changing the position of plate for the HPTLC method. Repeatability is reported in terms of relative standard deviation (RSD).

### Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of TAM (800, 1200 and 1600 ng per spot). The results are reported in terms of relative standard deviation (RSD).

### Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the both the drugs were found by visual inspection.

### Robustness of the method

Robustness of the proposed method was estimated by changing different conditions like mobile phase composition ( $\pm 0.2$  ml for each component), mobile phase volume varied  $\pm 3$  %, scanning wavelength  $\pm 1$  nm and  $R_f$  value and peak areas were measured after development of plate and % RSD was calculated. A concentration level of 1000 ng per spot was employed.

### Solution Stability

The stability of standard solutions was tested after 0, 6, 12, 24, 48 and 72 h of storage. The stability of the solutions was determined by comparing peak area percentage and peak purity at 1000 ng per spot.

### Specificity

The specificity of the method was ascertained by analyzing standard drugs and sample. The spots TAM in samples were confirmed by comparing the  $R_f$  and spectra of the spots with that of standard. The peak purity of TAM was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot..

### Analysis of TAM in Tablets

To determine the content of TAM in conventional tablets, 30 tablets were weighed, their mean weight determined and finely powdered. Powder equivalent to 10 mg of TAM was transferred into a 100 ml volumetric flask containing 20 ml methanol, sonicated for 30 min and diluted to the mark with methanol. The

resulting solution was centrifuged for 10min at 5,000 rpm and the supernatant was diluted with methanol to obtain  $100 \text{ ng } \mu\text{l}^{-1}$  of the drug.  $10 \mu\text{l}$  this solution was applied to the HPTLC plate at 1000 ng per spot and followed by development. Analysis was carried out in triplicate, peak areas were measured at 282 nm and sample concentrations calculated. The potential interference from excipients was also examined.

#### **Forced Degradation of Tamsulosin hydrochloride**

Decomposition studies were performed in solutions containing drug at a concentration of 1 mg/ml. Samples were withdrawn at suitable time intervals and subjected to HPTLC analysis. The drug was subjected under different stress conditions as follows:

##### **Acid and Base Induced Degradation**

1 ml of the methanolic stock solution was diluted with each of 0.1 N HCl and 1 N NaOH to 25 ml separately and was refluxed at  $60^\circ\text{C}$ .

##### **Oxidative Degradation**

1 ml of the methanolic stock solution was diluted with 30 % (v/v) of hydrogen peroxide to 25 ml. The solution was refluxed at  $60^\circ\text{C}$ .

##### **Wet heat degradation**

1 ml of the methanolic stock solution was diluted with distilled water 25 ml and was refluxed in a boiling water bath to assess wet heat degradation.

##### **Dry heat degradation**

For dry heat degradation the standard drug was placed in an oven at  $100^\circ\text{C}$  for 24 h.

##### **Photostability**

The photochemical stability of the drug was studied by exposing the solution to direct sunlight for 48 h kept on a terrace. For UV degradation analysis, the solution was exposed to UV radiation for 48 h.

## **RESULTS AND DISCUSSION**

### **Optimization of the Chromatographic Conditions**

The TLC procedure was optimized with a view to develop a stability indicating assay method used for the quantification of the TAM in pharmaceutical tablets. Both the pure drug and the degraded products were spotted on the TLC plate and run in different solvent systems. Initially, neat solvents such as methanol, ethanol, n-butanol, acetonitrile, chloroform, n-hexane, benzene and acetone in different ratios were evaluated as mobile phase. Initially chloroform-acetonitrile 3:0.2 (v/v), chloroform-acetone 2:0.1 (v/v), toluene-n-butanol 3:0.3 (v/v) were employed as mobile phase gave spot lacked of compactness and considerable less  $R_f$  value. 0.29  $R_f$  value of TAM was achieved with mobile phase toluene-methanol 9:0.5 (v/v) but less compact and diffused spot was observed. Sharp spot of TAM was obtained when triethylamine was added to this solvent system. Finally this mobile phase was optimized to achieve good peak symmetry and better separation of TAM from degradation peaks

obtained under different stressed conditions. The spot appeared to be more compact with a more symmetrical peak shape when TLC plates were pre-treated with methanol and activated at  $110^\circ\text{C}$  for 5min. Well-defined standard spots along with its degradation products were obtained when the chamber saturation time was optimized to 30min at  $25^\circ\text{C}$  temperature.

Good resolution and considerable high  $R_f$  value ( $0.71 \pm 0.02$ ) was obtained for TAM using optimized mobile phase toluene-methanol-triethylamine 9:3:1 (v/v/v) with densitometric scanning at 282 nm (Fig. 1).

### **Validation of the Method**

The calibration plot was linear over a concentration range of 100–2000 ng per spot. A good linear relationship was observed over this range ( $r^2 = 0.9973 \pm 0.0051$ , slope =  $2.093 \pm 0.0682$ , intercept =  $1679.34 \pm 0.481$ ). Repeatability of sample application and measurement of peak area was expressed as RSD and were 0.461 % and 0.363 % for six replicate determinations. The low values of RSD indicate that the proposed method is repeatable. The RSD value obtained for intra-day and inter-day variation were 0.752– 0.961 % and 0.848– 1.082 % respectively is low which indicates that proposed method is precise. RSD of peak areas during robustness studies were calculated for changes in parameters and were less than 2 % which indicates that method is robust and reproducible. LOD and LOQ values were found to be 10 and 50 ng per spot, respectively, and pointed towards adequate sensitivity of the method. Peak purity for TAM was assessed by comparing spectra acquired at the start, apex and end of the peak obtained from the scanning of spot, i.e.  $r(S, M)$  and  $r(M, E)$ . The high value of  $r$  indicates specificity of the method. Accuracy was determined on previously analysed formulations after spiking with 50, 100 and 150% of the additional drug. Mean recovery obtained is  $99.51 \% \pm 0.912$ . Validation parameters are summarized in Table 1.

### **Solution Stability**

There was no indication of degradation in solutions of TAM as revealed by peak purity data and from the value of RSD ( $< 2\%$ ) for peak areas of bands of solution stored at different times. The solution was found to be stable at ambient temperature for 72 h and no unknown peaks were observed.

### **Analysis of Formulated Tablets**

A single band was observed in samples extracted from tablets and there was no interference from the excipients which might have present in the tablets. The drug content was found to be 99.97 % with standard deviation 0.619. It was therefore inferred that degradation of TAM had not occurred in the marketed formulation analyzed by this method.

### Stability Indicating Properties

The chromatograms obtained after exposure of solution of TAM (1000 ng per spot) to acidic and alkaline conditions and oxidative conditions (Fig. 2, 3 & 4) showed well separated bands of TAM from additional bands of degradation impurities at different  $R_f$  values. For samples of same concentration were subjected to wet heat degradation, sun light, ultraviolet (UV) light at 254 nm and dry heat, no additional band was detected which indicated stability of TAM under these conditions. Spots obtained from degradation products were well resolved from the active ingredient. Peak purity of these spots of TAM resolved during stability study was assessed by comparing the respective spectra at peak start, peak middle and peak end, i.e.  $r(S, M)$  and  $r(M, E)$ . The peak purity data

indicated that peaks of TAM resolved after application of stress conditions were pure. All the degradation products obtained under different stress conditions are summarized in Table 2.

### CONCLUSION

The developed HPTLC procedure was precise, specific and accurate. Separation of TAM from degradation products confirmed stability indicating properties of this method. Statistical analysis indicated that the method was reproducible and selective for the analysis of TAM in bulk drug and in tablets without interference from excipients. This methodology may also be applied to the study of degradation kinetics and for its determination in plasma and other biological fluids.

**TABLE 1: SUMMARY OF VALIDATION PARAMETERS OF PROPOSED HPTLC METHOD**

Parameters	Value
Linearity & Range	100-2000 ng/spot
Correlation coefficient ( $r^2$ )	0.9973
Limit of detection	30 ng/spot
Limit of quantification	100 ng/spot
% Accuracy $\pm$ SD <sup>a</sup> (n=6)	99.51 % $\pm$ 0.912
Precision (% RSD <sup>b</sup> )	
Repeatability of sample application (n=6)	0.461
Repeatability of sample measurement (n=6)	0.363
Intraday (n=3)	0.752– 0.961
Interday (n=3)	0.848– 1.082
Robustness	Robust
Specificity	Specific
% Assay $\pm$ SD <sup>a</sup> (n=6)	99.97 % $\pm$ 0.619

<sup>a</sup>SD = Standard deviation, <sup>b</sup>RSD = Relative standard deviation

**TABLE 2. SUMMARY OF DEGRADATION PRODUCTS OF TAM UNDER DIFFERENT STRESS CONDITIONS**

Stress conditions	Rf values of degradation products	Peak purity data of TAM $r(S,M)$ , $r(M,E)$
Acid hydrolysis, 0.1N HCl, refluxed for 6 h at 60°C	0.50	0.9996, 0.9997
Alkaline hydrolysis, 1N NaOH, refluxed for 4 h at 60°C	0.81	0.9994, 0.9992
Oxidation (H <sub>2</sub> O <sub>2</sub> , 30% v/v), refluxed for 8 h at 60°C	0.55,0.62	0.9993, 0.9995
Wet heat, refluxed for 10 h at 100°C	Not detected	0.9997, 0.9994
Dry heat, heated in oven at 100°C for 24 h	Not detected	0.9998, 0.9996
Day light, exposed to sunlight for 48 h	Not detected	0.9994, 0.9998
UV light, exposed to UV light (254 nm) for 48 h	Not detected	0.9995, 0.9993

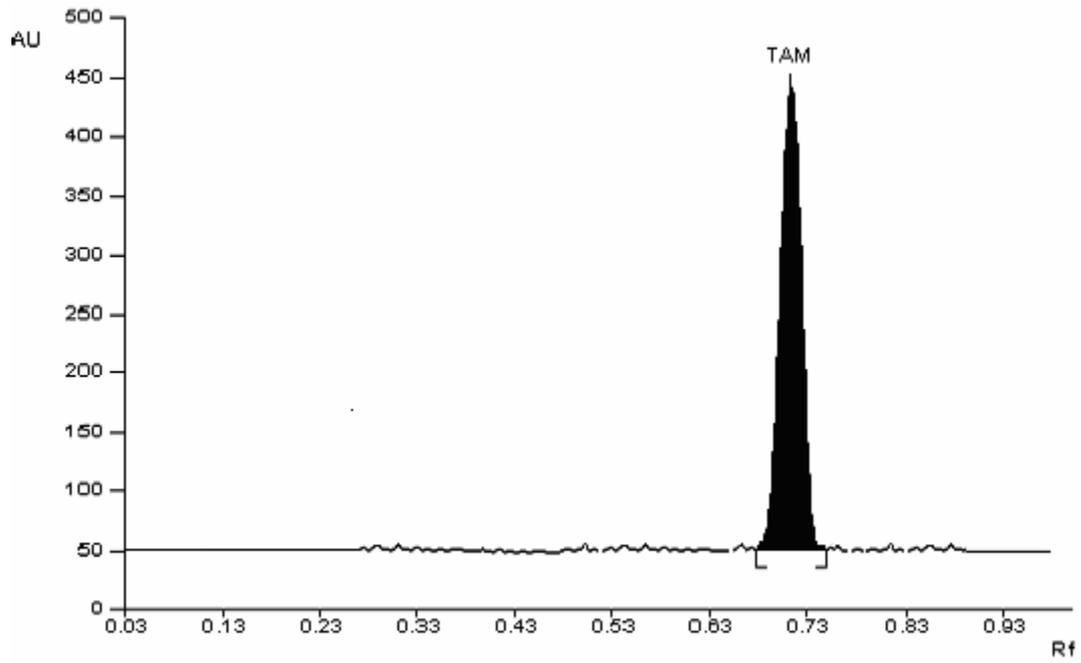


Fig. 1. A typical densitogram of standard Tamsulosin hydrochloride with Rf value  $0.71 \pm 0.01$

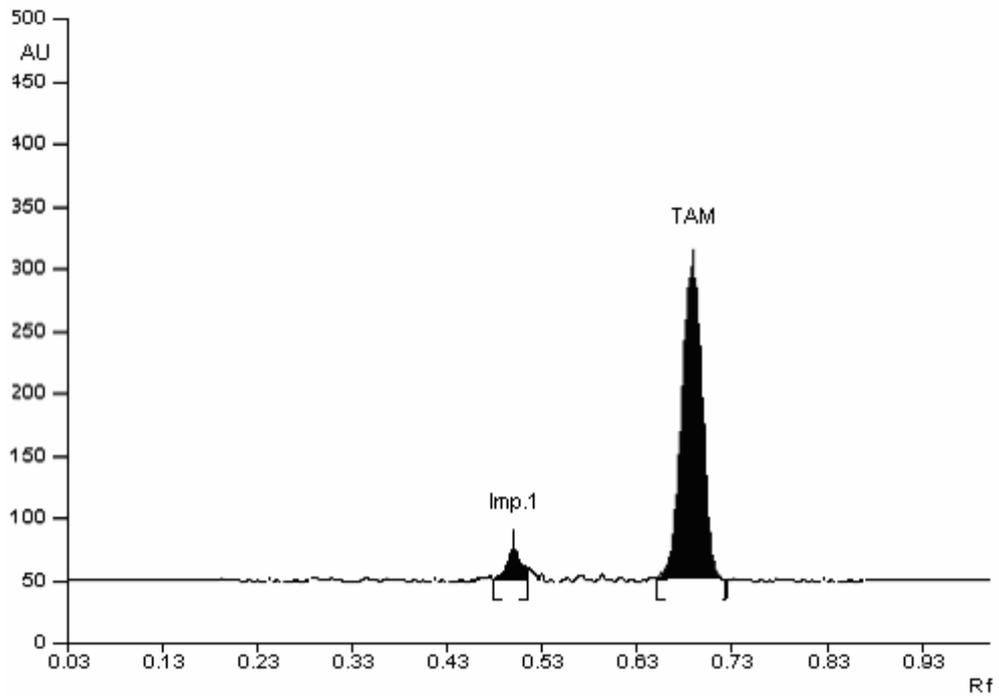
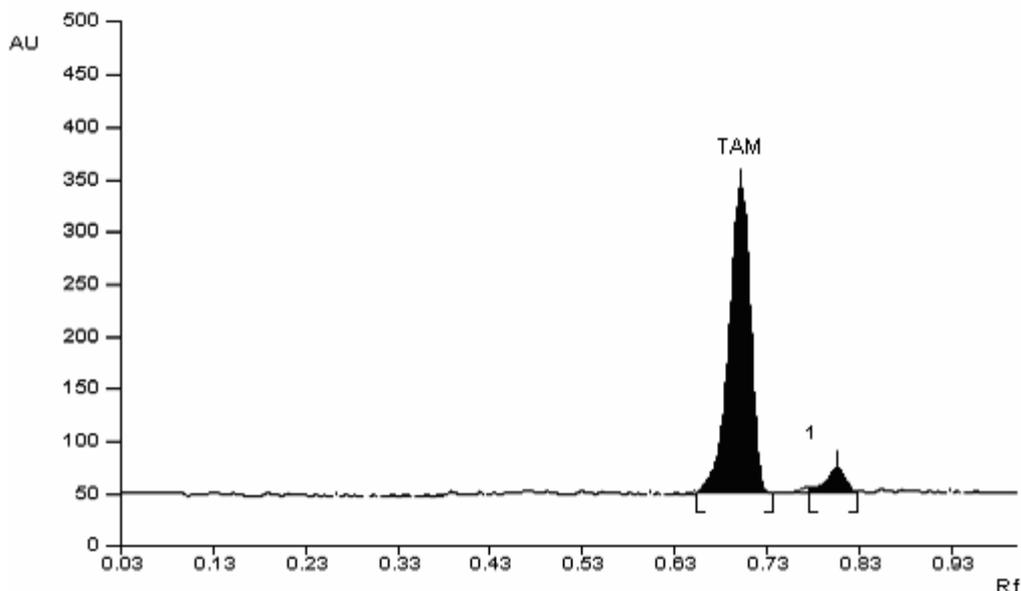
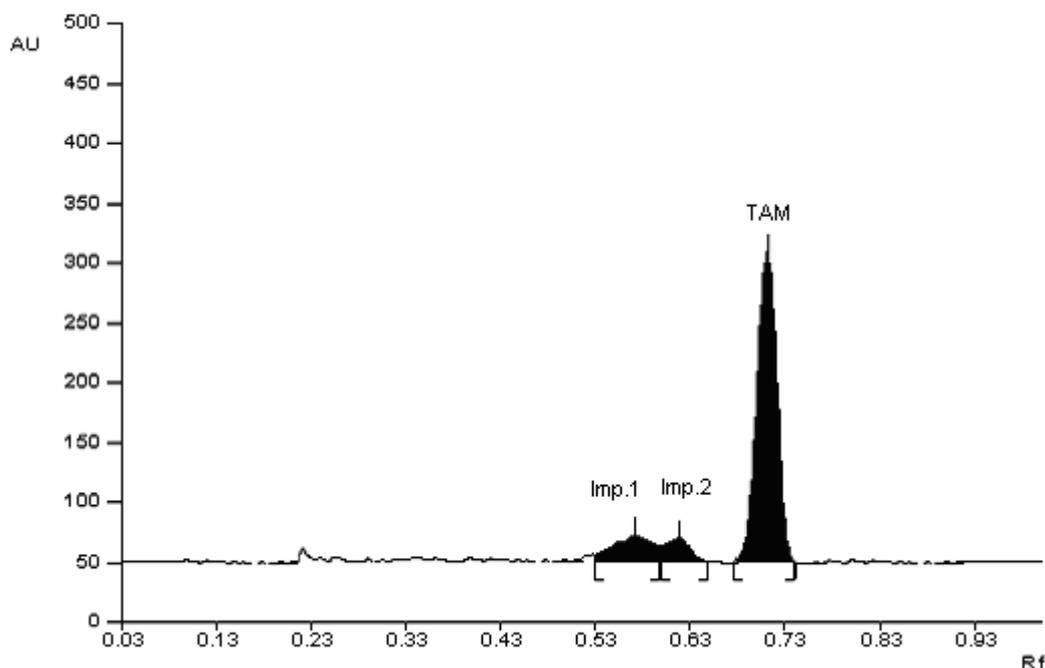


Fig. 2. Chromatogram obtained after acid degradation of Tamsulosin hydrochloride



**Fig. 3. Chromatogram obtained after alkaline degradation of Tamsulosin hydrochloride**



**Fig. 4. Chromatogram obtained after oxidative degradation of Tamsulosin hydrochloride**

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