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Method Development and Forced Degradation Studies of Atazanavir in Solid Dosage Form by RP-HPLC

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ABSTRACT

The proposed method was found to be simple and rapid for method development of Atazanavir from pure and its dosage forms. The mobile phase is simple to prepare and economical. Atazanavir was subjected to various stress conditions using acid, alkali, oxidative and photolytic degradation. The sample was injected into the HPLC system to identify the degradation products and to check whether the drug is stable when exposed to such conditions. From the forced degradation studies, it was found that Atazanavir was stable during acid degradation when compared to alkali, hydrogen peroxide and direct sunlight. During acid degradation, the retention time of Atazanavir was changed from 1.892 to 1.9 minutes and 0.8% drug was decomposed which is quite less when compared to other stress conditions. There is a wide scope for the development of new analytical methods for the assay of, "Atazanavir" by exploiting their characteristic physical and chemicals properties.

INTRODUCTION:

The term 'Chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase[1].Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or based on the size of the particles (e.g. Size exclusion chromatography).

Different modes of chromatography are as follows[2]:

- Normal Phase Chromatography
- Reversed Phase Chromatography
- Reversed Phase – ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography
- Size Exclusion Chromatography

High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result, the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

FORCED DEGRADATION STUDIES:

STRESS TESTING: Defined as the determination of the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedures used.

An intrinsic stability characteristic of drug molecule includes developing an understanding of

- Conditions leading to degradation
- Rates of degradation
- Chemical structure of the degradation products
- Degradation pathways.

Stress testing studies involve exposure of the drug substance to the stress conditions of heat, humidity, photo stress (UV and VIS), oxidative conditions, and aqueous conditions across a broad pH range. The intent is to induce 10-20% degradation of the parent drug. Without stress testing, there is no way to assess whether or not the method will resolve and detect the degradation products. In reality, RP-HPLC method with photodiode array UV detection and gradient elution to cover a wide polarity range.

Forced degradation studies can be carried out through following routes.

- Solvolysis
- Oxidation
- Photolysis
- Dehydration
- Racemization
- Incompatibilities

MATERIALS AND METHODS:

METHOD DEVELOPMENT AND OPTIMIZATION:

LIST OF INSTRUMENTS USED:

Sr. No.	Instruments/Equipments/Apparatus
1.	A SHIMADZU HPLC with Class-VP version 6.12 SP1 software, UV-Visible Detector (SPD-10A), PUMP (LC-10AT) and (LC-10ATvp).
2.	UV-Visible double beam Spectrophotometer (ELICO)
3.	Electronic Balance (AFCOSET)
4.	Ultra Sonicator (ENERTECH)

LIST OF CHEMICALS, REAGENTS AND STANDARDS:

Sr. No.	Chemicals / Reagents / Standards	Grade	Batch No	Specification
1	Tetra butyl ammonium hydrogen sulphate	AR	DL8S48123	99.5%
2	Methanol	HPLC	R191L04	99.7%
3	Acetonitrile	HPLC	R054B03	99.9%
4	Triple distilled water	NA	NA	NA
5	Atazanavir sample	NA	NA	99.94 (w/v)
6	Clopidogrel sample	NA	NA	99.99 (w/v)
7	Sodium Hydroxide	NA	NA	NA
8	Hydrochloric Acid	NA	NA	NA

OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR ATAZANAVIR:

Parameters	Conditions
Stationary phase (column)	C_{18} RP Column (250 mm x 4.6mm x 5 µm
Mobile Phase	Methanol: 5mM TBHS (50:50%v/v)
Flow rate (ml/min)	1.0 ml
Runtime (minutes)	8
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	299
Internal standards	Clopidogrel

PREPARATION OF MOBILE PHASE:

Methanol and 5mM Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) were properly mixed in the ratio of 50:50.

PREPARATION OF TETRA BUTYL AMMONIUM HYDROGEN SULPHATE (TBHS): 0.84885 gm of Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) was added to 500 ml of double distilled water to make 5 mM solution of TBHS.

PREPARATION OF STANDARD DRUG AND INTERNAL STANDARD SOLUTION:

Stock solution of Atazanavir (1 mg/ml) was prepared by dissolving 25 mg of Atazanavir in 25 ml of volumetric flask containing 10 ml of Methanol and 10 ml of 5 mM Tetra Butyl Ammonium Hydrogen Sulphate. The solution was sonicated for about 10 min and then made up to volume with mobile phase. Working standard solutions of Atazanavir was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly, stock solution of internal standard was prepared by dissolving 25 mg of Clopidogrel in 25 ml of volumetric flask containing 10 ml of Methanol and 10 ml of 5 mM Tetra Butyl Ammonium Hydrogen Sulphate, sonicated for 10 min, then made up to the volume with mobile phase. Working standard solutions of Atazanavir were prepared by taking suitable aliquots of drug solution from the standard stock solution of 1000 µg/ml, spiked with an internal standard solution (0.1 ml from 1000 µg/ml) and the volume was made up to 10 ml with mobile phase.

FORCED DEGRADATION STUDIES OF ATAZANAVIR:

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

Acid degradation:

About 10 mg of Atazanavir pure drug was accurately weighed and transferred to 10 ml volumetric flask. One ml of 0.1N HCl was added and kept aside for one hr and made up to volume with mobile phase. Then from this 10 mcg/ml solution was prepared and 20µl of the sample solutions was injected in HPLC system to obtain chromatogram.

Alkaline degradation:

About 10 mg of Atazanavir pure drug was accurately weighed and transferred to a 10 ml volumetric flask. One ml of 0.1N NaOH was added and kept aside for one hr and made up to volume with mobile phase. Then from this 10 mcg/ml solution was prepared and 20µl of the sample solutions were injected in HPLC system to obtain chromatograms.

Oxidative degradation:

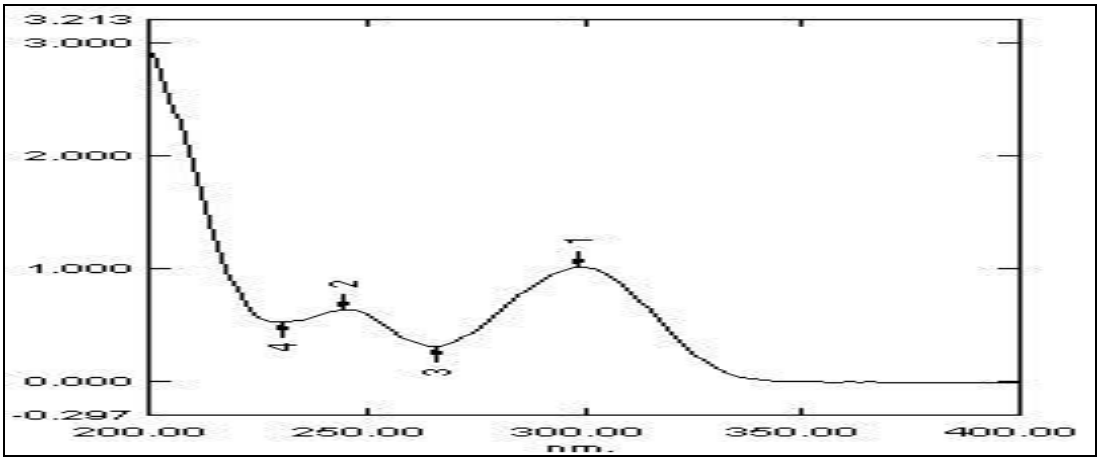
About 10 mg of Atazanavir pure drug was accurately weighed and transferred to three 10 ml volumetric flasks and one ml of 3% w/v of hydrogen peroxide were added and kept aside for two hrs and made up to volume with mobile phase. Then from this 10 mcg/ml solution was prepared and injected in HPLC system to obtain chromatogram.

Photolysis:

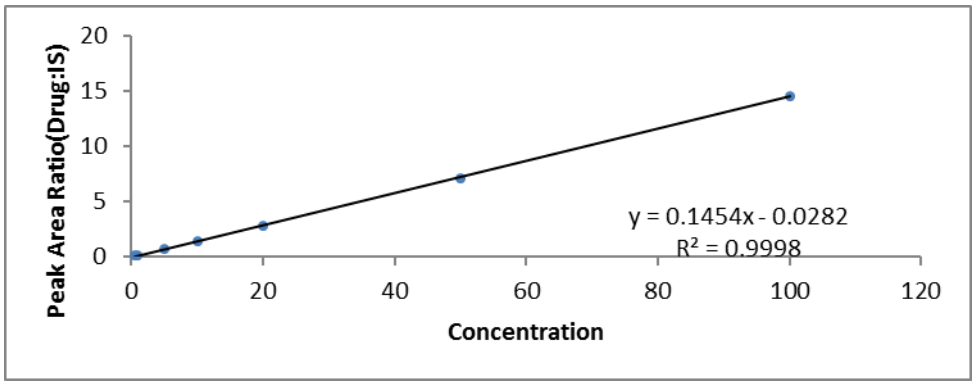
About 10 mg of Atazanavir pure drug was accurately weighed and transferred to 10 ml volumetric flask and made up to volume with mobile phase and kept aside under the direct sunlight. Then from this 10 mcg/ml solution was prepared and injected in HPLC system to obtain chromatograms.

RESULTS AND DISCUSSIONS:

UV SPECTRUM OF ATAZANAVIR:



METHOD DEVELOPMENT OF ATAZANAVIR:

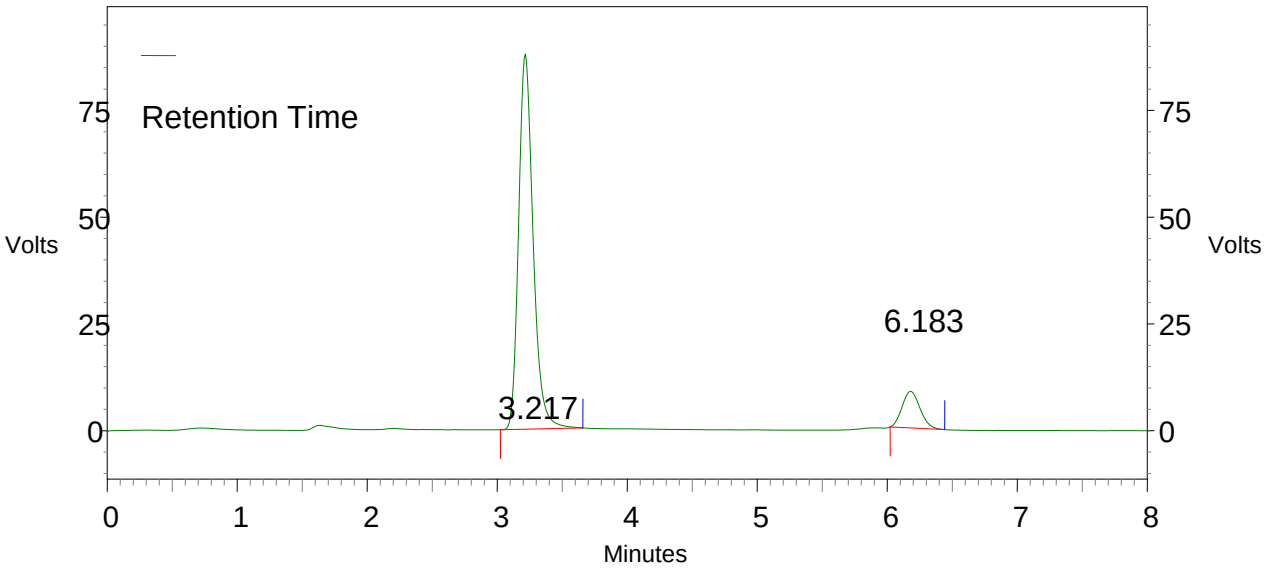


Linearity graph of Atazanavir

Formulation	Labelled amount (mg)	HPLC method*		
		Mean amount found (mg)	% Recovery by proposed method	% RSD
Atazor	100	100.97 ± 0.9013	100.9741 ± 0.9013	0.8926

Amount of Atazanavir present in Formulation

From the linearity table, it was found that the drug obeys linearity within the concentration range of 0.5-100 µg/ml for Atazanavir. From the results shown, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility.



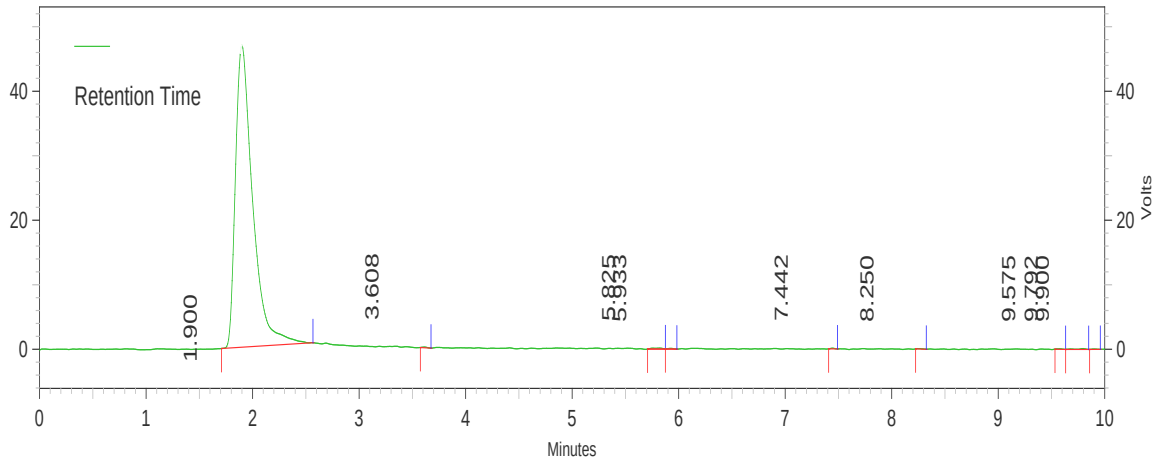
A Typical Chromatogram of Atazanavir (50µg/ml) and Clopidogrel (10µg/ml) in pure form

Sr. No.	Name of the Peaks	Retention time (min)
1.	Atazanavir	3.217
2.	Clopidogrel	6.183

FORCED DEGRADATION STUDIES OF ATAZANAVIR:

Atazanavir was subjected to various stress conditions using acid, alkali, oxidative and photolytic degradation. The sample was injected into the HPLC system to identify the degradation products and to check whether the drug is stable when exposed to such conditions.

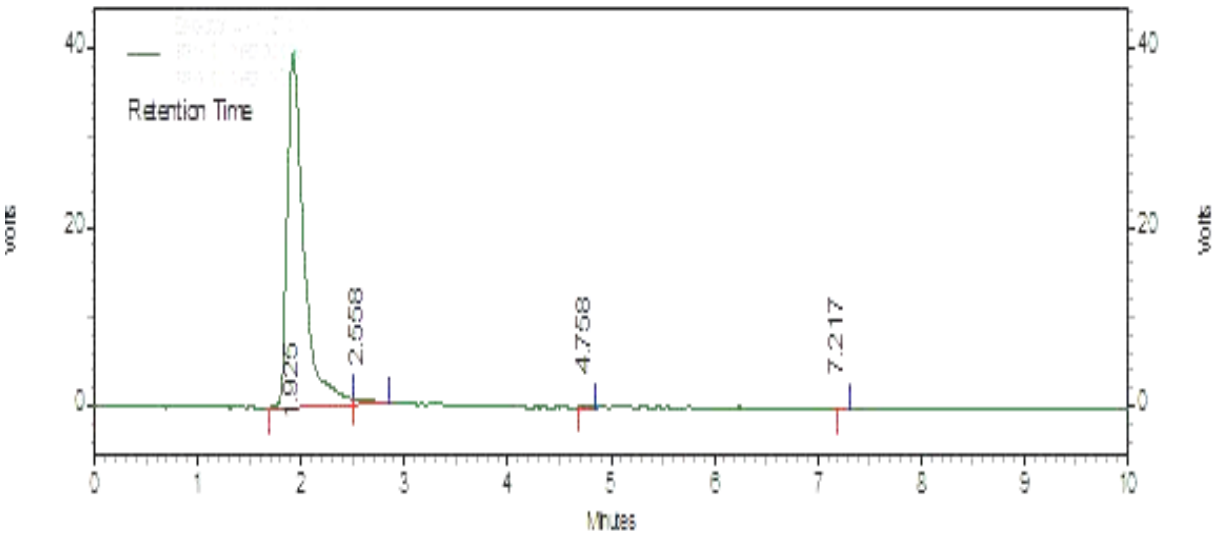
ACID DEGRADATION: 0.1 N HCl, 25ºC, 10µg/ml



Pk #	Retention Time	Area	Area %	Height	Height %
1	1.900	132539	99.568	9007	98.54
2	3.608	52	0.059	16	0.202
3	5.825	184	0.209	26	0.327
4	5.933	73	0.083	19	0.239
5	7.442	43	0.049	17	0.214
6	8.250	38	0.043	10	0.126
7	9.575	42	0.048	13	0.164
8	9.792	111	0.126	21	0.264
9	9.900	31	0.035	11	0.139
Totals		133113	100.000	9140	100.000

10µg/ml sample (drug) solution treated with 0.1 N HCl was injected after 1 hour into the HPLC system, the retention time was changed from 1.892 to 1.9 minutes and 0.8% drug was decomposed. The major degraded products were observed at 3.608, 5.825 and 9.792 minutes.

ALKALINE DEGRADATION: 0.1 NaOH, 25ºC, 10µg/ml

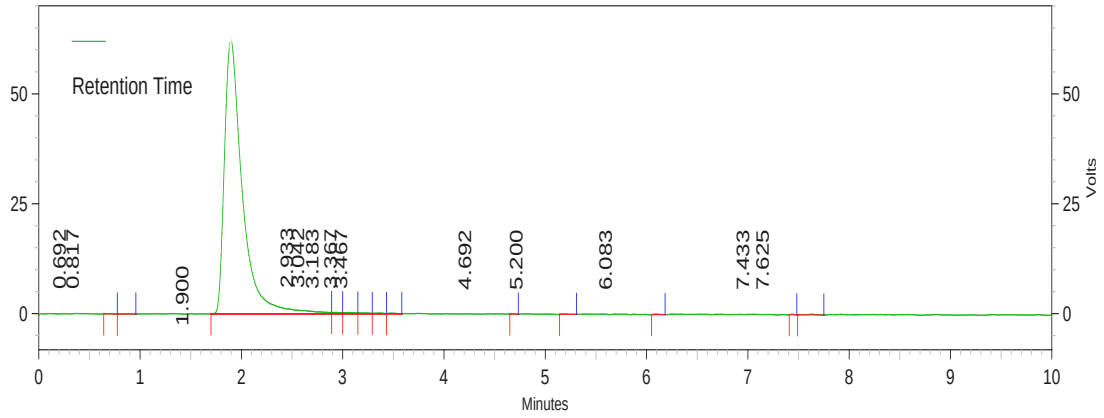


Detector A - 1

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.925	74014	98.330	6598	97.937
2	2.558	1087	1.444	104	1.544
3	4.758	102	0.136	17	0.252
4	7.217	68	0.090	18	0.267
Totals		75271	100.000	6737	100.000

10µg/ml sample (drug) solution treated with 0.1 N NaOH was injected after 1 hour into the HPLC system, the retention time was changed from 1.892 to 1.925 minutes and 44.61% drug was decomposed. The major degraded products were observed at 2.558 minutes.

OXIDATIVE DEGRADATION: 3% H_2O_2 , 25^0c, 10 µg/ml



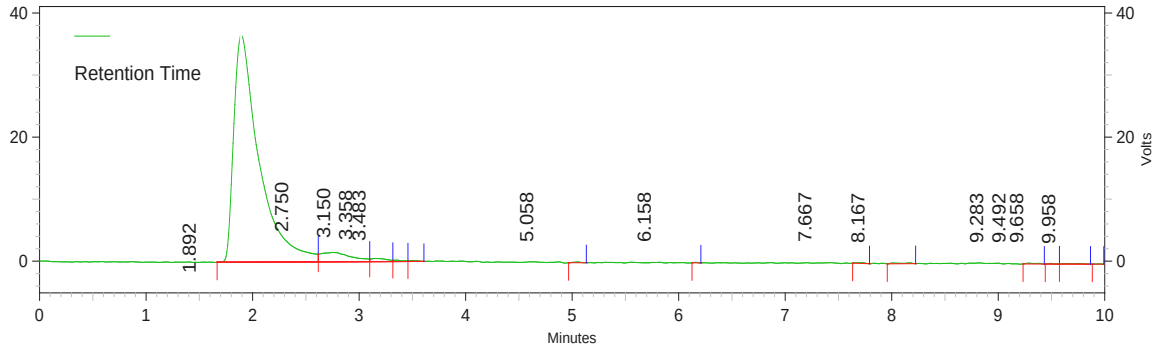
Detector A - 1

Pk #	Retention Time	Area	Area %	Height	Height %
1	0.692	59	0.046	14	0.131
2	0.817	114	0.089	18	0.169
3	1.900	126946	98.691	10377	97.263
4	2.933	296	0.230	51	0.478
5	3.042	354	0.275	47	0.441
6	3.183	224	0.174	31	0.291
7	3.367	164	0.127	26	0.244
8	3.467	107	0.083	20	0.187
9	4.692	45	0.035	17	0.159
10	5.200	56	0.044	13	0.122
11	6.083	71	0.055	18	0.169
12	7.433	34	0.026	14	0.131
13	7.625	160	0.124	23	0.216
Totals		128630	100.000	10669	100.000

10µg/ml sample (drug) solution treated with 3% H_2O_2 was injected after 1 hour into the HPLC system, the retention time was changed from 1.892 to 1.9 minutes and 4.96% drug was decomposed. The major degraded products were observed at 0.692, 2.933, 3.183, 3.367 and 7.625 minutes.

PHOTOLYSIS:

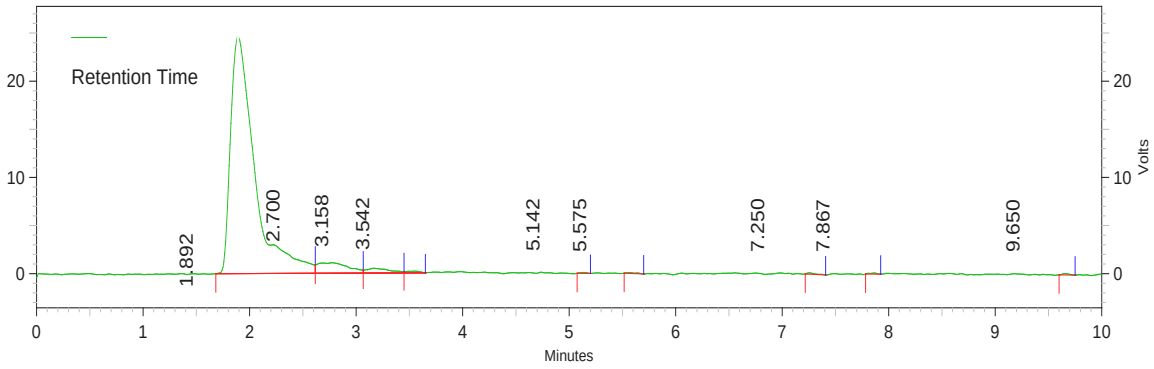
8 hours:



Detector A - 1

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.892	100205	93.425	6097	92.030
2	2.750	4931	4.597	250	3.774
3	3.150	863	0.805	85	1.283
4	3.358	228	0.213	38	0.574
5	3.483	133	0.124	22	0.332
6	5.058	93	0.087	20	0.302
7	6.158	35	0.033	13	0.196
8	7.667	131	0.122	18	0.272
9	8.167	200	0.186	22	0.332
10	9.283	149	0.139	20	0.302
11	9.492	62	0.058	17	0.257
Totals		107257	100.000	6625	100.000

16 hours:



Detector A - 1

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.892	63845	91.998	4106	91.488
2	2.700	3594	5.179	176	3.922
3	3.158	1230	1.772	83	1.849
4	3.542	261	0.376	30	0.668
5	5.142	69	0.099	17	0.379
6	5.575	93	0.134	12	0.267
7	7.250	110	0.159	22	0.490
8	7.867	95	0.137	20	0.446
9	9.650	101	0.146	22	0.490
Totals		69398	100.000	4488	100.000

10 µg/ml sample (drug) solution exposed to direct sunlight was injected after 8 hours into the HPLC system, the retention time was remained unchanged and 74.99% drug was decomposed. The major degraded products were observed at 2.75, 3.15, 3.358, 8.167 minutes. Similarly, 10 µg/ml sample (drug) solution exposed to direct sunlight was injected after 16 hours into the HPLC system, the retention time was remained unchanged and 52.3% drug was decomposed. The major degraded products were observed at 2.7, 3.158 minutes for 16 hr sample.

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for determination of Atazanavir from pure and its dosage forms. The mobile phase is simple to prepare and

economical. From the linearity table, it was found that the drug obeys linearity within the concentration range of 0.5-100 µg/ml for Atazanavir. From the results shown, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. From the forced degradation studies, it was found that Atazanavir was stable during acid degradation when compared to alkali, hydrogen peroxide and direct sunlight. During acid degradation, the retention time of Atazanavir was changed from 1.892 to 1.9 minutes and 0.8% drug was decomposed which is quite less when compared to other stress conditions. There is a wide scope for the development of new analytical methods for the assay of, "Atazanavir" by exploiting their characteristic physical and chemicals properties.

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REFERENCES:

1. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development.2nd ed. New York: Wiley-Interscience; 1997, pgs-686-712.
2. Patel T, Brutto RL. Method Validation. In: Yuri Kazakevich, Rosario Lo Bruto, editors. HPLC for Pharmaceutical Scientists. New Jersey: Wiley-Interscience; 2007, pgs-455.
3. S. Colombo, N. Guignard, C. Marzolini, A. Telenti, J. Biolla and L.A. Decosterd, "Determination of the new HIV-protease inhibitor Atazanavir by liquid chromatography after solid phase extraction", Journal of Chromatography, 2004; Vol.No. 810, pgs- 25-34.
4. Adrienne C Müller and Isadore Kanfer, "An efficient HPLC method for the quantitative determination of atazanavir in human plasma suitable for bioequivalence and pharmacokinetic studies in healthy human subjects", Journal of Pharmaceutical and Biomedical Analysis, 2010.
5. Estelle Cateau, Nicolas Tournier, Antoine Dupuis, Gwenaël Le Moal and Nicolas Venisse, "Determination of atazanavir in human plasma using solid-phase extraction and high- performance liquid chromatography", Journal of Pharmaceutical and Biomedical Analysis, 2005; Vol No. 39, Pg No. 791-795.
6. Rolf W. Sparidans, Frits Dost, Kristel M. L. Crommentuyn, Alwin D. R. Huitema, Jan H. M. Schellens, Jos H. Beijnen**, "**Liquid chromatographic assay for the protease inhibitor atazanavir in plasma", Biomedical Chromatography, 2005; Vol No.20, Pg No. 72-76.

7. Loyd. K. Snyder, Joseph. J. Kirkland and Joseph. L. Glajch, "Practical HPLC Method Development 2nd Edition, Wiley – Interscience Publication, USA Page No. 742-747.
8. Willard, H.Y. Merritt L.L., Dean J.A. and Settle FA "Instrumental Methods of Analysis", 7th Edition CBS Publishers and Distributors, New Delhi.1991 Page 436-439.
9. Pandeya SN.A Textbook of Medicinal Chemistry (Synthetic and Biochemical Approach). 3rd ed.Varanasi: SG publisher; 2003.