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Development and Validation of RP- HPLC Assay Method for Determination of Ibandronate Sodium in Tablet Dosage Form

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ABSTRACT

A simple, accurate and sensitive liquid chromatographic method has been developed for the assay of ibandronate sodium drug substance in tablet dosage form. The separation was achieved on Hypersil BDS C18 (250mm X 4.6mm), 5µm column. The mobile phase consisted of Buffer: ACN (95:05) v/v; flow rate 1.0 ml min^{-1} at ambient temperature. The analytes were monitored by PDA detector. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolytic, thermal and humidity degradation. Considerable degradation was achieved under thermal condition. Mass balance was demonstrated in all stress conditions. The method was validated for specificity, precision, linearity, solution stability and accuracy. The average recoveries for ibandronate were in the range of 99.0–102.0% and the method can be successfully applied for the routine analysis of ibandronate sodium drug substance.

Key words: RP- HPLC, Stability indicating, Ibandronate Sodium.

INTRODUCTION

Ibandronate sodium [(1-hydroxy-3-(methyl pentyl amino) propylidene bisphosphonic acid monosodium monohydrate)] is the sodium salt of ibandronic acid, a synthetic nitrogen-containing bisphosphonate drug. This new third generation bisphosphonate is used to treat patients with bone disease like Paget's disease, malignant hypercalcemia and postmenopausal osteoporosis (Sankar Babu VR *et al*., 2010). For quantification of impurities and assay of ibandronate sodium, few analytical methods have been reported. Indirect fluorescence detection was used in a high performance ion exchange chromatographic method based on the formation of the non-fluorescent Al3+-ibandronate complex after post-column addition of the fluorescent Al^{3+}-morin reagent (Narendra kumar M *et al*., 2011). Ibandronate was determined by high performance ion exchange chromatography with UV detection at 240nm after complex formation with Cu2+ ion. Ibandronate sodium was determined by capillary zone electrophoretic method within direct detection at 254nm, the limit of detection (LOD) values reported for ibandronate was 352–1760µgml^{-1}. The aim of this study was to develop a simple, sensitive, precise liquid chromatographic method with PDA detection for the determination of ibandronate sodium.

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Materials and Methods

Chemicals and Reagents:

The standard sample of ibandronate sodium drug substance was procured from Aarti Drugs Ltd, Boisar-Thane. Analytical reagent (AR grade) Disodium Edetate, Thomas Baker, Pentanesulfonic acid sodium salt HPLC Grade, Merck, Triethylamine HPLC Grade, Rankem, Orthophosphoric acid (OPA) HPLC Grade, Rankem, Water HPLC grade procured from Milli-Q system.

High Performance Liquid Chromatography

Agilent HPLC 1200 series chromatograph equipped with binary pump, 2695 Photodiode Array Detector with data processing capacity was used. A Hypersil BDS column C18 (250 mm x 4.6 mm, 5 mm) was used. The pH measurement was performed by using LAB INDIA- PICO controlled pH analyzer equipped with pH electrode. Mobile phase filtration was performed by vacuum pump using 0.45 µm filter paper. As a degasser, PCI Analytics Pathak ultrasonicator was used. Typical operating conditions include flow rate, 1 ml/min; injection volume, 10 µl; wavelength, 200nm; column compartment temperature, 35^0C; and operating condition, room temperature. The retention times of the ibandronate

sodium peak is at about 26.79 min, respectively. Relative standard deviation for the peak areas of the six replicate injections for ibandronate peak is not more than 1.0% (Table.1).

Table.1 Assay Results of Ibandronate Sodium

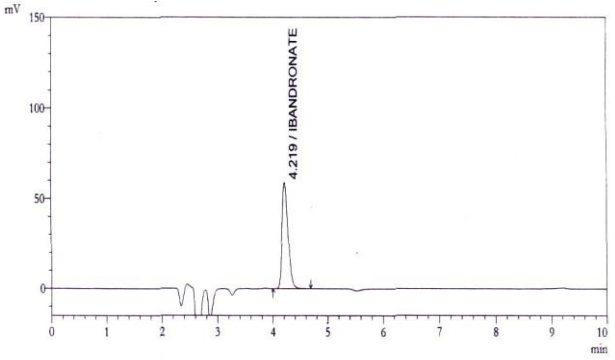
Ibandronate Sodium 150 mg Tablet	
RT	4.21 min
Area	STD (A_s)-444924
	SPL(A_T)- 432008
Weight of Standard in mg (W_s)	64 mg
Weight of Sample in mg (W_T)	2105.0
Theoretical Plates	8124
% Assay of IBN Na (P)	94.4%
Label Claim of IBN Na	150 mg
Molecular Weight	341.21gm/mol
Average Weight of Tablet in mg (A_w)	527.6
Limits for Assay (%)	98-102
Assay by Practically (%)	99.8

Preparation of Standard and Sample Solutions

Preparation of standard solutions

Weigh accurately and transfer 64mg of Ibandronate sodium (working standard) to 25 mL volumetric flask. Add about 15 mL diluent and sonicate to dissolve. Allow to equilibrate to room temperature and make up volume with diluent, mix. Dilute 5 mL of the solution to 20 mL with diluent, mix. Final concentration of Ibandronate sodium is 640 ppm (figure.1).

Figure.1 Overlaid Standard Chromatogram of Ibandronate Sodium



Sample Preparation

Weigh accurately and transfer 8 intact tablets to 500 mL of volumetric flask. Add about 50 mL of diluent and sonicate to disperse. Add about 300 mL of diluent and sonicate for 25 min with intermittent shaking. Allow to equilibrate to room temperature and dilute to volume with diluent, mix. Centrifuge the solution at 2500 RPM for 5 min. dilute 5 mL of supernatant to 20 mL with diluent, mix. Filter through 0.45 nylon filter discarding first 3 mL of the filtrate (Figure.2).

Method validation

Linearity study

In order to prepare stock solution, 256 mg Ibandronate sodium was accurately weighed, dissolved in diluent with sonication and diluted to 100 ml with the diluent. The mobile phase was filtered through 0.45-µm membrane filter and delivered at 1ml/min for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 200 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

Figure.2 Overlaid Sample Chromatogram of Ibandronate Sodium

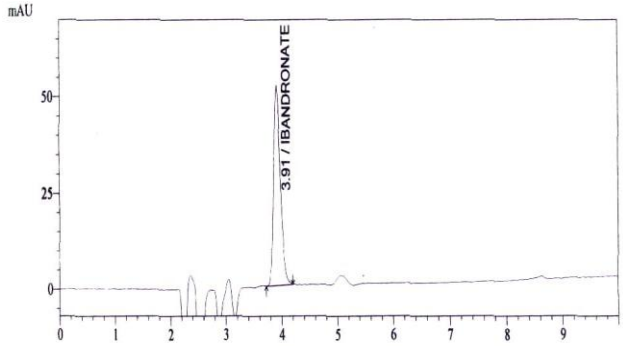


Figure.3 Linearity of Ibandronate Sodium

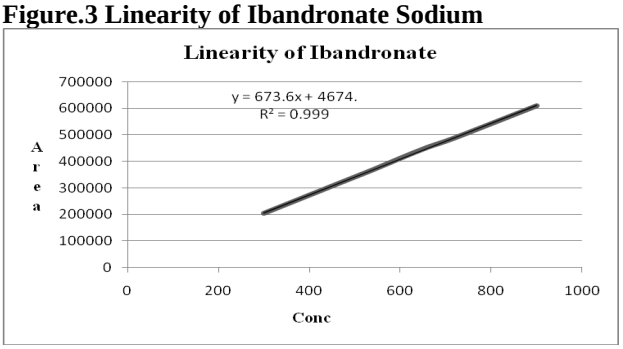
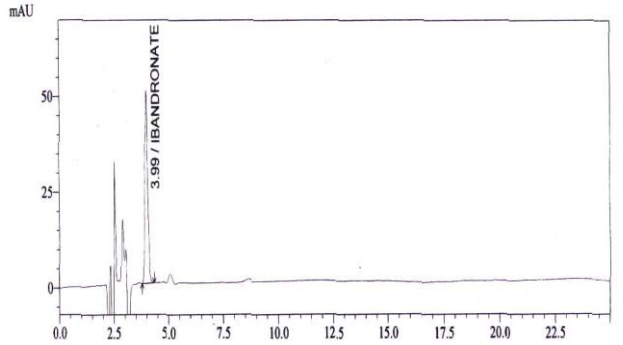


Figure.4 Overlaid Chromatogram of Acid Stressed Sample



Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is also termed as trueness. It was done by recovery study. Sample solutions were prepared with 100% in triplicate.

System Precision (Repeatability)

Repeatability expresses the precision under the same operating conditions over a short interval of time.

Repeatability is also termed intra-assay precision. Solutions of Ibandronate Sodium were prepared as per test method and injected for 6 times. The mean SD and RSD were checked for precision.

Intermediate precision (Ruggedness)

Six samples were prepared by different analyst by using different column, different system on different day. The system suitability criteria were evaluated. % RSD of for above 6 preparations was calculated and the overall % RSD for above experiment results was also calculated.

Analytical solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of standard and sample. The standard and sample were prepared and injected into HPLC at initial and different time intervals up to 24 hrs and cumulative % RSD for peak area was determined.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Range to be inferred from the data of linearity, recovery and precision experiments.

Specificity and selectivity

Specificity is the ability to asses unequivocally the analyte in the presence of components which maybe expected to present. The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample. The method is quite selective. There was no other interfering peak around the retention time of Ibandronate Sodium also the baseline did not show any significant noise.

Forced Degradation Studies

Acid degradation studies

10 tablets were weighed. The average weight was determined. Sample powder equivalent to 100 mg of Ibandronate sodium was transferred in to a 100 ml volumetric flask. About 50 ml diluent was added and sonicated for 10mins. Added 5 ml of 5M HCl and kept at 80°C on water bath for 5 hrs. Then solution was allowed to cool at room temperature, 5 ml of 5M NaOH solution was added, for neutralization, and volume was made up to the mark with diluent and mixed properly.

These solutions were centrifuged and subsequent solutions were collected. A 10 µl of these solutions were injected into LC, under optimized chromatographic conditions (Figure.4).

Alkali degradation studies

10 tablets were weighed. The average weight was determined. Sample powder equivalent to 100 mg of Ibandronate sodium was transferred in to a 100 ml volumetric flask. About 50 ml diluent was added and sonicated for 10mins. Added 5 ml of 5M NaOH and kept at 80°C on water bath for 5 hrs. Then solution was allowed to cool at room temperature, 5 ml of 5M HCL solution was added, for neutralization, and volume was made up to the mark with diluent and mixed properly. These solutions were centrifuged and subsequent solutions were collected. A 10 µl of these solutions were injected into LC, under optimized chromatographic conditions (Figure.5).

Figure.5 Overlaid Chromatogram of Base Stressed Sample

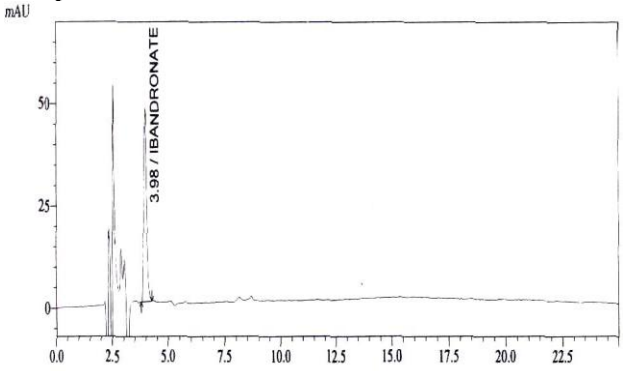


Figure.6 Overlaid Chromatogram of Peroxide Stressed Sample

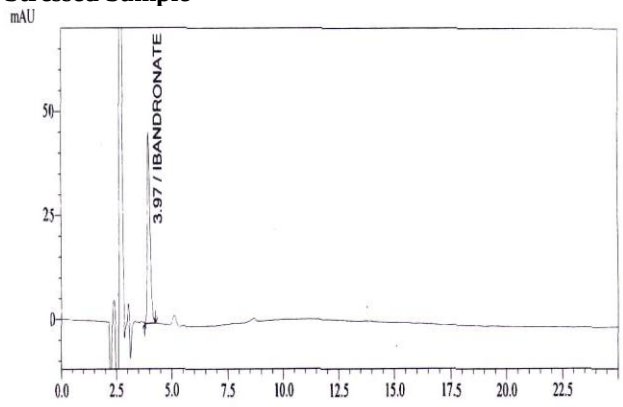


Figure.7 Overlaid Chromatogram of Heat Stressed Sample

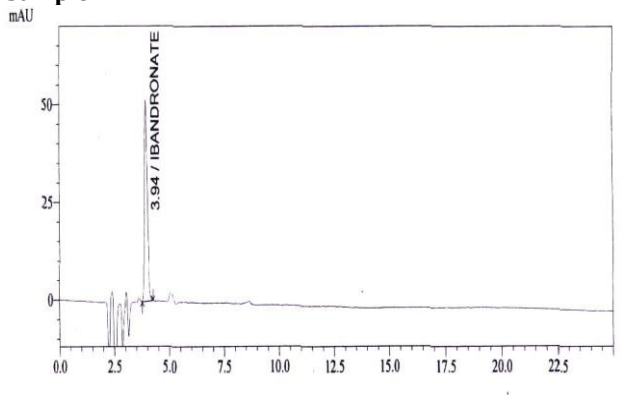


Figure.8 Overlaid Chromatogram of Humidity Sample

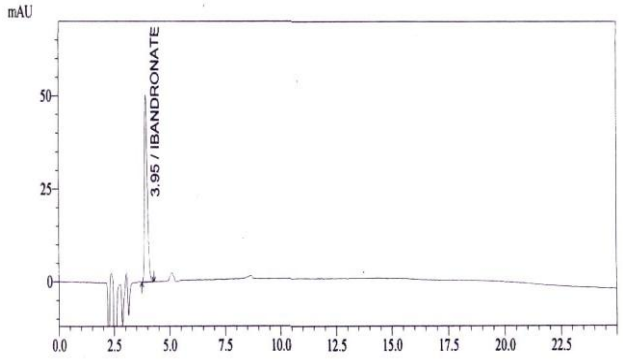


Figure.9 Overlaid Chromatogram of UV Stressed Sample

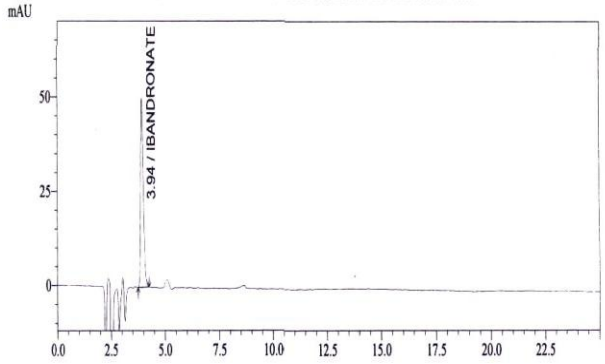


Table.2 Statistical Data of Ibandronate Sodium

Statistical Parameters	Ibandronate
Correlation Coefficient	0.99
Slope	673.6
Concentration Range	300-900 ppm

Oxidation studies

Weighed and determined average weight of 10tablets. Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask. About 50 ml diluent was added and sonicated for 10mins. 5 ml of 3% H_2O_2 was added and kept at 80°C for 5 hrs, equilibrated to room temperature and made up to volume with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions (Figure.6).

Temperature stress studies/ Dry heat induced-degradation

Crushed tablet content was heated at 105°C, for 24 hrs and allowed to cool to room temperature. Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask, 75 ml of diluent was added and sonicated for 10mins and volume was made up to the mark with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions (Figure.7).

Humidity degradation

Tablet was kept in 40°C/75% RH chamber for 24 hrs. Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask, about 75 ml of diluent was added and sonicated for 10mins and made up to volume with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions (Figure.8).

Photostability studies

Crushed tablet powder was kept in the photo stability chamber and exposed to, as per ICH guidelines (An overall illumination of not less 1.2 million lux hrs and an integrated near ultraviolet energy of not less than 200 watt hrs/sq m). Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask, about 75 ml of diluent was added and sonicated for 10mins and made up to volume with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions (Figure 9).

RESULTS AND DISCUSSION

Method Development and optimization

As there is no chromophore present in ibandronate sodium, there was no possibility for UV or fluorescence detection and no suitable groups are present for derivatization. Ibandronate sodium; for this reason water was chosen as diluents. Preliminary experiments were carried out Using Hypersil BDS C18 Column with Buffer: ACN (70:30) v/v (adjusted to pH 2 using OPA) Ibandronate was lost peak shape while on Inertsil ODS Column, with Buffer: ACN (40:60) v/v peak was tailed with asymmetry 2.41.Ibandronate was determine on Hypersil BDS C18 column, peak was separated using phosphate buffer with pH 7.0 (1.75gm pentanesulfonic acid sodium salt+100mg EDTA in 900ml of water +6ml TEA, dilute upto 1000ml, adjust pH with OPA); Buffer: ACN (95:05) v/v. Better resolution obtained using acetonitrile as organic modifier. Satisfactory separation and good peak shapes were achieved within a reasonable time using a mobile phase of 95:5% (v/v) mixture of buffer and ACN with a flow rate of 1.0 ml min^{-1}. The effect of column temperature on separation was studied at different temperatures ranging from 35^0C to 65^0C. Ambient temperature was found to be optimal from the point of view of both resolution and peak shape.

Method validation

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment.

Linearity

The linearity of photodiode array detector response of ibandronate sodium at different concentrations was studied in the range 300-900 $\mu g ml^{-1}$

for ibandronate sodium. The data was subjected to statistical analysis using a linear-regression model. The regression equations for ibandronate sodium is y = 673.6x+ 4674. The statistical parameters slope and correlation coefficient values were calculated and shown in Table.2 and 3.

Table.3 Results of Linearity of Ibandronate Sodium

Sample Name	PPM	Area I	Area II	Area III	Mean	SD	%RSD
50% Level	300	203091	205961	204955	204669	1456	0.71
80 % Level	480	328026	328038	327244	327769	455	0.14
90 % Level	540	366035	368650	370846	368510	2409	0.65
100 % Level	600	411370	411535	411834	411580	235	0.06
110 % Level	660	448987	457880	451345	452737	4607	1.02
120% Level	720	489559	487680	487680	488306	1085	0.22
150 % Level	900	608060	608499	609448	608669	709	0.12

Table.4 Results Accuracy of Ibandronate Sodium

Levels	Spiked Spl (mg)	Area I	Area II	Area III	Mean	Recovery	% Recovery
100 %	574.20	366299	366299	366299	366299	574.36	100.0
100 %	574.30	364985	364985	364985	364985	572.30	99.7
100 %	574.10	365375	365375	365375	365375	572.92	99.8

Accuracy

Accuracy of method was determined by recovery experiments using standard addition technique. Recoveries were determined by adding the ibandronate sodium in triplicate, i.e.100% recovery values are given in Table.4.

System Precision (Repeatability)

Solutions of Ibandronate sodium were prepared as per test method and injected for 6 times. The mean SD and RSD were checked for precision. Results are shown in Table. 5a.

Intermediate precision (Ruggedness)

% RSD of for above 6 preparations was calculated and the overall % RSD for above experiment results was also calculated. Results are shown in Table.5b.

Table.5a Results of System Precision (Reproducibility)

System Precision	
Injection	**Area of Ibandronate**
1	443650
2	432008
3	447833
4	438202
5	450637
6	445838
SD	98.7± 1
%RSD	1.30

Table.5b Results of Intermediate Precision

Ruggedness Study	
Overall ±SD	0.48
Overall % RSD	0.72

Table.6a Analytical Solution Stability

Standard	Ibandronate Standard		Ibandronate Sample	
Time	**Area**	**% Diff. w.r.t. Initial**	**Area**	**% Diff w.r.t. Initial**
Initial	403821		443650	
1 hr	406938	-0.8	441588	0.5
4 hrs	401320	0.6	440750	0.7
8 hrs	398905	1.2	440823	0.6
12 hrs	399105	1.2	440482	0.7
16 hrs	401538	0.6	439141	1.0
20 hrs	400368	0.9	439305	1.0
24 hrs	403034	0.2	440194	0.8

Table. 6 b

Cumulative Mean	Cumulative SD	Cumulative % RSD
1232.2	**18.07**	**0.52**

Analytical solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of standard and sample. The standard and sample were prepared and injected into HPLC at initial and different time intervals up to 24 hrs. Results are shown in Table.6a, b).

Table.7 Evaluation of Forced Degradation Studies

Condition	Ibandronate Sodium	% Degradation w.r.t. control
Control Sample	422017	-
Acid stressed sample	380991	9.72
Base stressed sample	390876	7.37
Peroxide stressed sample	407199	3.51
Heat stressed sample	378297	10.35
Humidity stressed sample (40^0c/75)	411059	2.59
UV Stressed sample	418970	0.72

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Range to be inferred from the data of linearity, recovery and precision experiments it was found to be 300-900 µgm/ml.

Forced Degradation Study:

Force degradation study carried out to evaluate the stability indicating properties of method. This study was performed with six different stressed conditions i.e acid hydrolysis, base hydrolysis, oxidation, heat stressed, humidity stressed and UV stressed. In this study, thermal degradation is obtained upto 10.35% which is higher than all other conditions. In case of UV stressed sample drug is stable than other conditions.

Assay Results of Tablet Dosage Form

The % Assay was found to be 99.8 ± 0.25 of ibandronate sodium in tablet dosage forms.

Summary and Conclusion:

A simple, accurate and sensitive liquid chromatographic method has been developed for the assay of ibandronate sodium drug substance in tablet dosage form. The separation was achieved on Hypersil BDS C18 (250mm X 4.6mm), 5µm column. The mobile phase consisted of Buffer: ACN (95:05) v/v; flow rate 1.0 ml min^{-1} at ambient temperature. The analytes were monitored by PDA detector. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolytic, thermal and humidity degradation. Considerable degradation was achieved under thermal condition. Mass balance was demonstrated in all stress conditions. The method was validated for specificity, precision, linearity, solution stability and accuracy. The average recoveries for ibandronate were in the range of 99.0–102.0% and the method can be successfully applied for the routine analysis of ibandronate sodium drug substance.

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