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Reverse phase high performance liquid chromatography method development and validation for estimation of etoricoxib and thiocolchicoside in pure and pharmaceutical dosage form

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ABSTRACT

A new, simple, Accurate, precise, robust and rugged reverse phase-HPLC method was developed for the simultaneous estimation of the Etoricoxib and Thiocolchicoside in pure and pharmaceutical dosage forms. Chromatogram was run through Hypersil C18 (250 mm×4.6 mm, 5µm) particle size. Mobile phase containing Potassium dihydrogen phosphate (0.03M) (pH-2.8): Methanol (75:25%) was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at Ambient. Optimized wavelength selected was 226 nm. Retention time of Etoricoxib and Thiocolchicoside were found to be 1.693min and 3.235min ± 0.02 respectively. The precision %RSD of the Etoricoxib and Thiocolchicoside were and found to be 0.435 and 0.039 respectively. %Recovery was obtained as 100.06% and 100.083% for Etoricoxib and Thiocolchicoside respectively. Regression equation of Etoricoxib is y = 48138x + 5396.0., and y = 71.91x + 42.07 of Thiocolchicoside. The LOD and LOQ values were found to be for the Etoricoxib and Thiocolchicoside are 1.27µg/ml, 1.16 µg/ml 3.81µg/ml, 3.48µg/ml and the proposed method was found to be simple, precise, accurate, rapid, economic and reproducible for the estimation of Etoricoxib and Thiocolchicoside in pure form and pharmaceutical marketed formulation.

Keywords: Etoricoxib and Thiocolchicoside, HPLC, Method Development, Validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure. Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1] Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life. There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This

happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

Different methods of analysis

The following techniques are available for separation and analysis of components of interest.

Spectral methods: The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods: Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods: Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques: Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques: GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma-Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry). Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc. are mentioned in Table 1.[2]

Table1: Summary of Hyphenated separation techniques.[2]

Separation technique	Hyphenated mode
Liquid chromatography	Liquid chromatography-mass spectrometry(LC/MS) Liquid chromatography-Fourier-transform infrared Spectrometry(LC-FTIR) Liquid chromatography-nuclear magnetic resonance spectroscopy(LC/NMR) Liquid chromatography-inductively coupled plasma mass spectrometry(LC-ICPMS)
Gas chromatography	Gas chromatography-mass spectrometry(GC/MS) Gas chromatography-Fourier-transform infrared(GC-FTIR) Gas chromatography-FTIR-MS(GC-FTIR-MS)
Capillary electrophoresis	Capillary electrophoresis-mass spectrometry(CE/MS) Capillary electrophoresis- nuclear magnetic resonance spectroscopy(CE/NMR) Capillary electrophoresis-surface enhanced Raman spectrometry (TLC-SERS)
Thin layer chromatography (TLC)	Thin layer chromatography- mass spectrometry (TLC/MS) Thin layer chromatography- surface enhanced Raman spectrometry (TLC-SERS)

Superficial fluid chromatography/ extraction (SFC/SFE)	Superficial fluid extraction-capillary gas chromatography-mass spectrometry (SFE-CGC-MS) Superficial fluid-Fourier-transform infrared (SFC-FTIR)

MATERIALS AND METHODS

Ortho-Phosphoric Acid from Finar, Acetonitrile HPLC from Merck, Methanol HPLC from Merck, Water HPLC from LobaChemi, Potassium dihydrogen orthophosphate AR from Finar, Etoricoxib from Sura Labs, Thiocolchicoside from Sura Labs.

Optimized chromatographic method

Optimized Chromatographic parameters:

Mobile phase : Potassium dihydrogen phosphate (0.03M) (pH-2.8): Methanol (75:25)

Auto sample temperature: Ambient
Injection volume : 20µL
Column: Hypersil C18 (250 mm×4.6mm,5µm)particle size
Detector wavelength : 226 nm
Flow rate : 1.0ml/min
Run time : 6 minutes

Procedure:

Inject 20µL of standard, sample into chromatographic system and measure the areas for the Etoricoxib and Thiocolchicosidepeeks and calculate the % assay by using the formula.

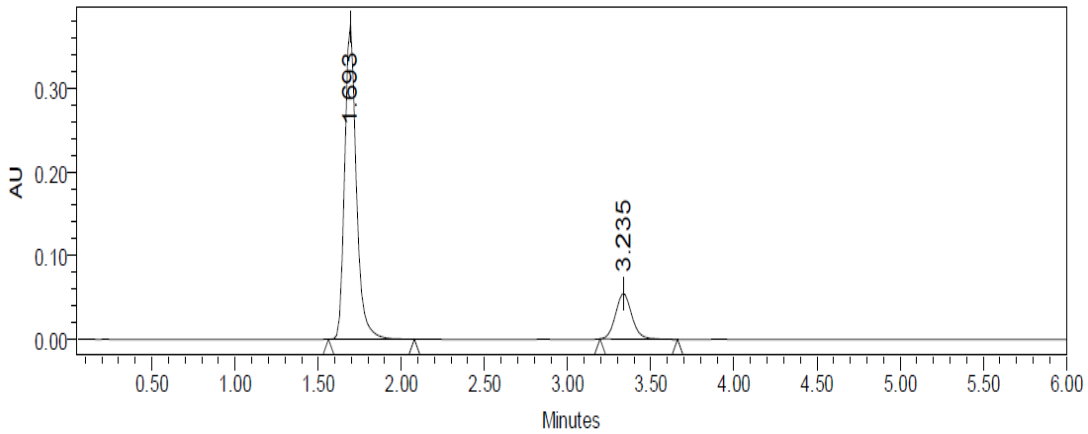

Fig 1: Typical Chromatogram for optimized method

Table 2: Results of optimized method Chromatogram

S. No.	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1	Etoricoxib	1.693	3658985		1.58	5698
2	Thiocolchicoside	3.235	6529	7.28	1.63	7529

Peeks are well separated all the parameters are within the limits.

Preparation of mobile phase

Transfer 1.36086gof Potassium dihydrogen phosphate into1000ml of beaker and adjust pH 2.80 with orthophosphoric acid (OPA).

Transfer the above solution 750ml and 250ml of methanol is used as mobile phase. They are mixed and sonicated for 20minutes.

Preparation of the etoricoxib and thiocolchicosidestandard and sample solution

Preparation of standard solution

Accurately weigh and transfer 50 mg of Etoricoxib and 50 mg ofThiocolchicoside into 50 ml of volumetric flask and add 10ml of water and sonicate 10min (or) shake 5min and make with water.

Further pipette out 0.8ml of Etoricoxib and 0.9ml of Thiocolchicoside from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of sample stock solution

Commercially available six tablets ware weighed and powdered the powdered equivalent to the 585.58 mg of Etoricoxib and Thiocolchicosideof active ingredients were transfer into a 50 ml of volumetric flask and add 10ml of methanol and sonicate for 20min (or) shake 10 min and make up with water.

Further pipette out 0.8ml of Etoricoxib and 0.9ml of Thiocolchicoside from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

RESULTS AND DISCUSSION

System suitability

Tailing factor for the peaks due to Etoricoxib and Thiocolchicoside in standard solution should not be more than 2.0. Theoretical plates for the Etoricoxib and Thiocolchicoside peaks in standard solution should not be less than 2500.

Table 3: System suitability data of Etoricoxib and Thiocolchicoside

parameter	Etoricoxib	Thiocolchicoside	Acceptance criteria
Retention time	1.691	3.299	_
Theoretical plates	5698	7529	>2500
Tailing factor	1.58	1.63	<2.00
% RSD	0.02	0.03	<2.00

Table 4: Standard Results of Etoricoxib

S. no	Sample name	RT	Area	USP plate count	USP tailing
1	Injection 1	1.694	3658986	5698	1.58
2	Injection 2	1.689	3659844	5655	1.59
3	Injection 3	1.692	3659864	5682	1.58
4	Injection 4	1.688	3654875	5674	1.58
5	Injection 5	1.688	3654514	5628	1.59
Avg.			3657617		
SD			2693.969		
% RSD			0.073654		

Table 5: Standard Results of Thiocolchicoside

S. no	Sample name	RT	Area	USP plate count	USP tailing
1.	Injection 1	3.244	6598	7598	1.63
2.	Injection 2	3.238	6574	7549	1.64
3.	Injection 3	3.246	6523	7561	1.63
4.	Injection 4	3.265	6539	7592	1.63
5.	Injection 5	3.265	6578	7569	1.64
Avg.			6562.4		
SD			30.59902		
% RSD			0.466278		

Results of system suitability study are summarized in the above table. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Specificity

Solution of standard sample and placebo were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria:

Chromatogram of standard and sample should be identical with near retention time.

Blank interference:

A study to establish the interference of blank was conducted. Solvent was injected into HPLC system as per the test procedure.

Acceptance criteria:

Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

Table 6: Specificity data for Etoricoxib and Thiocolchicoside

S. no	Sample name	Etoricoxib		Thiocolchicoside	
		Area	Rt	Area	Rt
1	Standard	3658985	1.691	6529	3.299
2	Sample	3785984	1694	6695	3.234
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Chromatograms explain that retention time for standard, sample and commercial product of Etoricoxib and Thiocolchicoside are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

Accuracy/recovery

Recovery study can be performed in the concentration range of 50% to 150% of the target concentration of the test. Minimum 3 concentrations are recommended. The average percentage recovery was between 97-103% and relative standard deviation of these recovery concentrations was less than 2%.

Table 7: Accuracy data for Etoricoxib

S. no	Accuracy level	Injection	Sample area	Rt
1	50%	1	1928492	1.687
		2	1935674	1.691
		3	1927546	1.688
2	100%	1	3859865	1.688
		2	3865143	1.688
		3	3858748	1.688
3	150%	1	5785847	1.686
		2	5786423	1.685
		3	5789658	1.684

Table 8: Accuracy data for Thiocolchicoside

S. no	Accuracy level	Sample name	Sample area	Rt
1	50%	1	3287	3.277
		2	3278	3.275
		3	3282	3.266
2	100%	1	6516	3.265
		2	6518	3.265
		3	6529	3.265
3	150%	1	9749	3.268
		2	9758	3.268
		3	9746	3.266

Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 100.00% for Etoricoxib and 100.00% for Thiocolchicoside. All the results indicate that the method is highly accurate**.**

PRECISION

Preparation of sample: Transfer the 802.04mg of sample into a 100ml of volume at flask and add 10ml of water and 10ml of methanol and sonicate 20min and makeup with water. Transfer the above solution into 5ml into 25ml volume metric flask dilute to the volume with water. The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.

Acceptance criteria: The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention time are not more than 2.0.

Table 9: Precision studies for Etoricoxib and Thiocolchicoside

S. no	Intraday precision for Etoricoxib			Intraday precision for Thiocolchicoside		
	Peak area	Mean peak area	%RSD	Peak area	Mean peak area	%RSD
1	3658952			6598		
2	3659854			6529		
3	3659874	3665965	0.435	6537	6547	0.390
4	3658748			6538		
5	3698547			6546		
6	3659816			6534		

Results of variability were summarized in the above table. The %RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

Linearity

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

Statistical Evaluation

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation coefficient, slope and, y-intercept were calculated.

Table 10: Linearity data for Etoricoxib

S. no	Concentration (µg/ml)	Rt	Area
1.	40	1.689	1923835
2.	60	1.691	2899874
3.	80	1.692	3868985
4.	100	1.689	4835984
5.	120	1.688	5758747

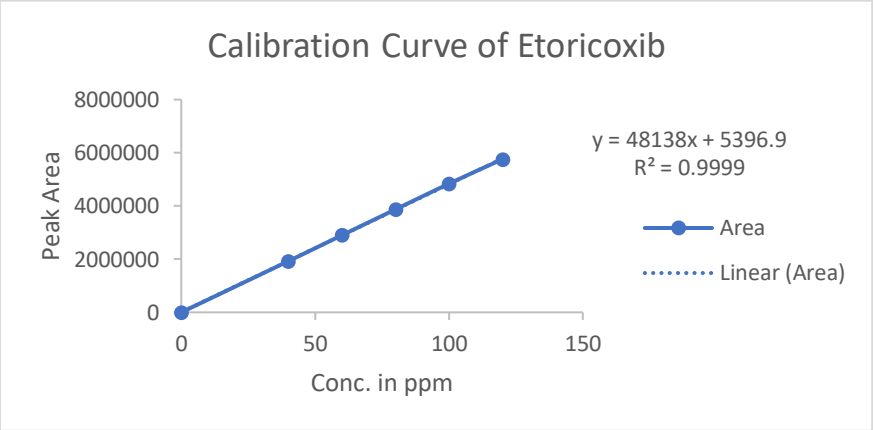

Fig 2: Linearity plot of Etoricoxib

Table 11: Linearity data for Thiocolchicoside

S. no	Concentration (µg/ml)	Rt	Area
1.	50	3.203	3675
2.	70	3.299	5108
3.	90	3.294	6529
4.	110	3.290	7954
5.	130	3.288	9349

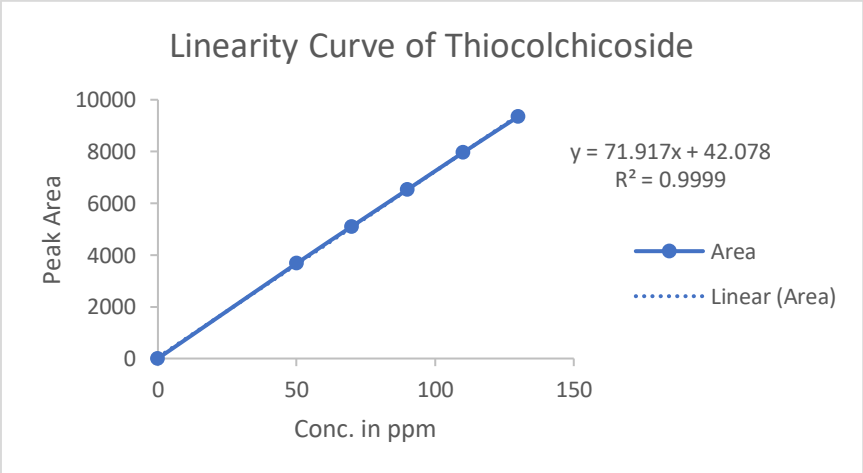

Fig: 3 Linearity plot of Thiocolchicoside

A linear relationship between peak areas versus concentrations was observed for Etoricoxib and Thiocolchicoside in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for Etoricoxib and 1 for Thiocolchicoside which prove that the method is linear in the range of 50% to 150%.

Robustness

Effect of variation in flow rate: Prepare the system suitability solution as per the test method and inject into the HPLC system with ± 0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

Effect of variation in Temperature: Prepare the system suitability solution as per the test method and injected into the HPLC with ± 5º C of the method temperature. Evaluate the system suitability values as required by the test method for both temperatures.

Table 12: Robustness data for Etoricoxib

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	1.868	5854	1.56
Increased flow rate (1.2ml/min)	1.544	5365	1.57
Decreased temperature (20^0c)	1.731	5418	1.53
Increased temperature (30^0c)	1.675	5496	1.54

Table 13: Robustness data for Thiocolchicoside

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	3.621	7598	1.62
Increased flow rate(1.2ml/min)	2.998	7612	1.61
Decreased temperature (20^0c)	6.242	7251	1.64
Increased temperature (30^0c)	2.302	7195	1.61

The results of robustness of the present method had shown that changes made in the flow and temperature did not produce significant changes in analytical results which were presented in the above table. As the changes are not significant we can say that the method is robust.

Limit of detction

The sensitivity of measurement of Etoricoxib and Thiocolchicoside by use of proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level.

LOD= 3.3 σ / S
Where,
σ = standard deviation of intercepts of calibration curves.
S = mean of slopes of the calibration curves.
The slope S may be estimated from the calibration curve of the analyte.
Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD

LOD = 3.3* σ/S
Where;
σ = standard deviation of response.
S = slope of calibration curve.
LOD for Etoricoxib = 1.27
LOD for Thiocolchicoside = 1.16

Limit of quantification

The sensitivity of measurement of Etoricoxib and Thiocolchicoside by the use of proposed method was estimated in terms of limit of quantification (LOQ). The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.
LOQ = 10 σ / S
Where,
σ = standard deviation of intercepts of calibration curves.
S = mean of slopes of the calibration curves.
The slope S may be estimated from the calibration curve of the analyte.
Minimum concentration of standard component in which the peak of the standard gets detected and quantification
LOQ = 10*σ/S
Where;
σ = standard deviation of the response.
S = slope of the calibration curve.
LOQ for Etoricoxib = 3.81
LOQ for Thiocolchicoside = 3.48

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative simultaneous estimation of Etoricoxib and Thiocolchicoside in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are

directly used without any preliminary chemical derivatisation or purification steps. Etoricoxib was found to be freely soluble in methanol, tetrahydrofuran, dimethyl sulfoxide, methyl ethyl ketone, dimethyl formamide, and chloroform. Etoricoxib is soluble in isopropyl acetate, ethanol and toluene, sparingly soluble in 2-propanol, and practically insoluble in water. Thiocolchicoside was found to be soluble in water, methanol, 0.1N HCl, 0.1N NaOH. Potassium dihydrogen phosphate (0.03M) (pH-2.8): Methanol (75:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine simultaneous determination of Etoricoxib and Thiocolchicoside in bulk drug and in Pharmaceutical dosage forms.

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