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SIMULTANEOUS ESTIMATION OF ETODOLAC AND PARACETAMOL IN BULK DRUG DOSAGE FORM BY RP-HPLC

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Abstract

A simple, specific, rapid, economical and accurate reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Etodolac and Paracetamol in bulk drug and tablet dosage form. Separation was achieved by cap cell pack C-18 column having 250 mm× 4.6 mm i.d. in isocratic mode, with mobile phase containing 25 nM potassium dihydrogen phosphate buffer (adjusted to pH 6.1 using ortho phosphoric acid) : methanol (40:60). The flow rate was 1.0 ml/min and effluents were monitored at 235 nm. The retention time of Etodolac and Paracetamol were 3.19 min and 6.08 min respectively. The linearity for Etodolac and Paracetamol were in the range of 10-100 µg/ml. The recoveries of Etodolac and Paracetamol were found in the range of 99.27-99.78 % and 99.74-99.88 % respectively. The proposed method was validated as per ICH and USP guidelines and successfully applied to the estimation of Etodolac and Paracetamol in bulk drug and tablet dosage form.

Keywords: Etodolac, Paracetamol, RP-HPLC Method, Validation

1. Introduction

1.1 Analytical Chemistry[1-5]

Analytical chemistry is the analysis of material samples to gain anunderstanding of their chemical composition and structure.During last few decades, analytical chemistry has witnessed extensivedevelopment in terms of sophistication, quantitation and Instrumentation. Consequently, newer analytical techniques (such as hyphenated techniques FTIR,GCMS, LCMS, HPLC, HPTLC etc.) and their areas of application have increased considerably because of the stringent requirements for testing and monitoring of thedrugs for approval; the demand on quality, validation data and performance of analytical methods have gained an importance. Analytical chemistry requires broad background knowledge of chemical and physical concepts. These hypermedia documents contain links to the fundamental principles that underlay the different analytical methods. With a fundamental understanding of analytical methods, a scientist faced with a difficult analytical problem can apply the most appropriate techniques. A fundamental understanding also makes it easier to identify when a particular problem cannot be solved by traditional methods, and gives an analyst the knowledge that is needed to develop creative approaches or new analytical methods. During the development of new drug product, detailed chemical studies must be made of raw materials, synthetic intermediates, the drug substance itself and the final formulated product. The set of analytical procedures developed to control the quality of the final marketed product must include both qualitative and quantitative methods in order to assure the identity and purity of the product. Qualitative analysis reveals the chemical identity of the analytes. Quantitative analysis gives the amount of one or more of these analytes in numerical terms. Therefore, qualitative information is required before a quantitative analysis.

1.2 Different Methods of Analysis: Analytical chemistry can be split into two main types,

A) **Qualitative analysis**

B) **Quantitative analysis**

A) Qualitative analysis: Qualitative inorganic analysis seeks to establish the presence of a given element or inorganic compound in a sample.[6] Qualitative analysis in chemistry focuses on a complete, detailed description of substances, processes, etc., in an attempt to get a thorough picture of what's going on. Mathematical descriptions are not generally part of qualitative analysis; instead, other observations are used to describe chemical properties and processes. This sort of analysis picks up otherwise-unclassifiable data, especially ambiguous data. But it is often not possible to extend the qualitative analysis description of one sample to other samples, largely because it is difficult or impossible to test findings of this nature. Typically, qualitative analysis by itself will not be done, but instead will be paired with quantitative analysis. Qualitative analysis makes a very good precursor for quantitative analysis; with a qualitative analysis, you can get a good idea of the items you want to later examine with a quantitative analysis.[7, 8]

B) Quantitative analysis

Quantitative analysis seeks to establish the amount of a given element or compound in a sample.

Quantitative Analysis is classified into two types:

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I) Classical methods: In Classical methods analysis were carried out by separating the component of interest in a sample by precipitation, extraction or distillation. For quantitative analysis the amount of analyte was determined by gravimetric or titrimetric measurements.

Volumetric Method: It is based on the determination of a solution of known strength required to complete a chemical reaction with the substance being analyzed.

Gravimetric Method: In this method, the assay results generally are obtained either by determining the weight of a substance in the sample, or the weight of some other substance derived from the sample, the equivalent weight of which serves as the basis for calculating the result.

II) Instrumental methods: Instrumental methods involves measurement of physical properties of analyte such as conductivity, electrode potential, light absorption or emission, mass to charge ratio began to be used for quantitative analysis of variety of inorganic, organic and biochemical analyte. Further more highly efficient chromatographic, electrophoresis technique began to replace distillation, extraction and precipitation for separation of components of complex mixtures prior to their qualitative or quantitative determination. Thesenewer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. These instruments vary in their principle method of detection as well in their objectives like quantitative and qualitative analysis. The method of analysis will also depend on their physical and chemical characters. Thermal sensitive products are not analyzed by thermal methods of analysis. Out of all these methods UV spectroscopy, FTIR, liquid chromatography, mass spectroscopy, etc. is used most frequently because they are easy for method development, operation, etc. Along with the method development its validation should be done for determination of analytical method consistency.[9, 10, 11]

1.3 Introduction to Chromatography[12,16]: Chromatography was discovered by M. S. Tswett in 1903 and can be described as a separation process that is achieved by distribution of substance between two phases, a stationary phase and mobile phase. Those solutes distributed preferentially in the mobile phase will move more rapidly through the system than those distributed preferentially in the stationary phase. Thus, the solute will elute in order of their increasing distribution coefficient with respect to the stationary phase. The basic principle behind chromatography method can be state as different distribution of the analytes between mobile and stationary phase results in different migration velocities.

Chromatography is probably the most powerful and versatile analytical technique available to the modern chemist. Its versatility comes from its capacity to handle a very wide variety of samples can range in complexity from a single substance to multicomponent mixture containing widely differing chemical species. In analytical chemistry it is prime important to gain information about the qualitative and quantitative composition of substances and chemical species that is to find out what substance is composed of and exactly how much. Separation of two sample components in chromatography is based on their different distribution between two non-miscible phases. The one is the stationary phase, a liquid or solid, is fixed in the system. The other is the mobile phase, a fluid, is streaming through the chromatographic system.

1.4 High Performance Liquid Chromatography

HPLC is high resolution, high pressure and high speed liquid chromatography. It has several times resolving power than open column liquid chromatography hence it is used for speedy resolution of complex mixture, separation and determination of species in a variety of organic, inorganic and biological materials.[9]

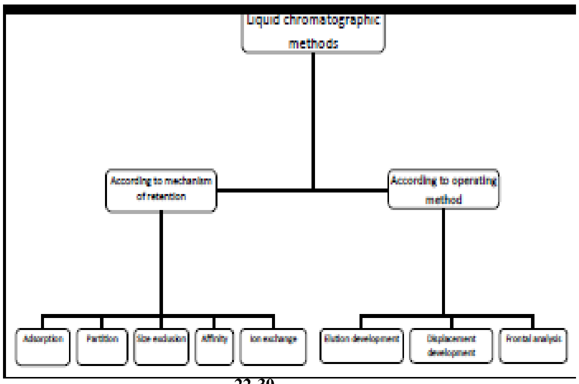
1.4.1 History of HPLC[17]

During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thinlayer chromatography. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and fast HPLC began to immerge. The past decade has seen a vast undertaking in the development of the microcolumns, and other specialized columns. The dimensions of the typical HPLC column are: 30 mm in length with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 µm to 200 µm. Fast HPLC utilizes a column that is shorter than the typical column, with a length of about 3 mm long, and they are packed with smaller particles.

1.4.2 Classification of Liquid Chromatographic Methods [18-21]

There are two ways to classify liquid chromatographic methods. Classification according to mechanism of retention classification according to operating method

Fig. 1: Schematic drawings of a six-port Rheodyne valve



1.4.3 HPLC theories[22-30]

Two main aspects comprise HPLC theory **Kinetic aspect** of chromatographic zone migration is responsible for the band broadening **Thermodynamic aspects** are responsible for the analyte retention in the column.

1.4.4 Basic Concepts of HPLC[23]

Chromatography is described and measured in terms of four major concepts: capacity, efficiency, selectivity, and resolution.

Dead time (t0): It is the time required by an inert compound to migrate from column inlet to column end without any retardation by the stationary phase. Consequently, the dead time is identical with the residence time of the sample compound in the mobile phase.

Net retention time (tR1 or tR2): It is the difference between total retention time and dead time. That is the time the sample component remains in the stationary phase.

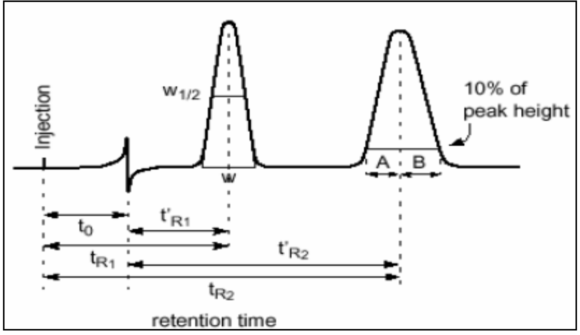
$$t'_{R_1} = t_{R_1} - t_0 \qquad t'_{R_2} = t_{R_2} - t_0$$

Tailing factor (T): It is a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as the tailing becomes more pronounced.

$$T = \frac{B}{A}$$

Where, A and B are determined by 10 % of peak height.

Fig. 2: Instrumental parameter of chromatography.



Capacity factor (k'): For effective liquid chromatographic separations, a column must have the capacity to retain samples and the ability to separate sample components, efficiently. The capacity factor, k'R, of a column is a direct measure of the strength of the interaction of the sample with the packing material and is defined by the expression-

$$k'_R = \frac{t_R - t_0}{t_0} = \frac{V_R - V_0}{V_0}$$

Where tR is the time taken for a specific solute to reach the detector and to is the time taken for nonretained species to reach the detector. VR is the volume of solution that is pumped through the detector before a specific peak is eluted, and V0 is the volume of solvent pumped through the detector between the time of injection and the appearance of the nonretained species. The capacity factor of a column is mostly a function of the packing material but can be manipulated to a degree by varying the solvent strength. The higher the capacity factor of the column, the greater is its ability to retain solutes

Selectivity (α): The selectivity of the chromatographic system is a measure of the difference in retention times between two given peaks and describes how effectively a chromatographic system can separate two compounds. Selectivity is usually defined in terms of α, where

$$\propto = \frac{t_2 - t_0}{t_1 - t_0} = \frac{V_2 - V_0}{V_1 - V_0} = \frac{k'_2}{k'_1}$$

The selectivity of a column is primarily a function of the packing material, although the chromatographer has some control using the mobile phase or temperature.

Resolution (R): Resolution is a term used to describe the degree of separation between neighbouring solute bands or peaks. It is affected by the selectivity (a), efficiency (N) and capacity (k') of the column. The resolution equation describes the relationship between those factors and indicates how they can be manipulated in order to improve the resolution between two peaks.

$$R = \frac{1}{4}\,\frac{\propto - 1}{\propto}\,(N^{\frac{1}{2}})\,\frac{k'}{1+k'}$$

Efficiency: Column efficiency refers to the performance of the stationary phase to accomplish particular separations. This entails how well the column is packed and its kinetic performance. The efficiency of a column, N, is a number that describes peakbroadening as a function of retention and is dependent on the entire chromatographic system.

$$n = 16\left(\frac{t_{R_1}}{w}\right)^2 \quad \text{or} \quad n = 5.54\left(\frac{t_{R_1}}{w_{1/2}}\right)^2$$

Height equivalent of a theoretical plate (h): HETP is the length, in which the chromatographic equilibrium between mobile and stationary phase is established. Since a large number of theoretical plates is desired, h should be as small as possible.

$$h = \frac{L}{n}$$

Two theories have been developed to describe the efficiency of the column: The plate theory

The rate theory The plate theory assumes that an instantaneous equilibrium is set up for the solute between the stationary and mobile phases, and it does not consider the effects of diffusional effects on column performance. The rate theory avoids the assumption of an instantaneous equilibrium and addresses the diffusional factors that contribute to band broadening in the column, namely, eddy diffusion, longitudinal diffusion, and resistance to mass transfer in the stationary phase and the mobile phase. The experimental conditions required to obtain the most efficient system can be determined by constructing a van Deemter plot.

1.4.4 Elution types

Isocratic elution: A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic elution. In this elution, peak width increases with retention time linearly according to the equation for N, the number of theoretical plates. This leads to the disadvantage that late-eluting peaks get very flat and broad. Their shape and width may keep them from being recognized as peaks. In isocratic elution, the selectivity does not change if the column dimensions change.

Gradient elution: A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower peaks for most components. This also improves the peak shape for tailed peaks, as the increasing concentration of the organic eluent pushes the tailing part of a peak forward. This also increases the peak height, which is important in trace analysis. The elution order may change as the dimensions or flow rate change.

1.4.5 Instrumentation for high performance liquid chromatography [26-30]

The basic components of a high-performance liquid chromatographic system consists of a) eluent containers for the mobile phase, b) a pump to move the eluent and sample through the system, c) an injection device to allow sample introduction, d) a column(s) to provide solute separation, e) a detector to visualize the separated components, f) a waste container for the used solvent, and g) a data collection device to assist in interpretation and storage of results.

Mobile phase reservoir, filtering system: The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with the special caps, Teflon tubing and filters to connect to the pump inlet and to the purge gas used to remove dissolved air. Helium purging and storage of the solvent under helium was found not to be sufficient for degassing of aqueous solvents. It is useful to apply a vacuum for 5-10 min. and then keep the solvent under a helium atmosphere.

Pumps: High pressure pumps are needed to force solvents through packed stationary phase beds. However, many separation problems can be resolved with larger particle packing that requires less pressure. Modern pumps have the following parameters: Flow rate range: 0.01 to 10 ml/min, Flow rate stability: not more than 1%, For SEC flow rate stability should be less than 0.2%, Maximum pressure: up to 5000 psi (345 bar, 340 atm)

The pumps are categorized as –

Constant displacement pump: It is a low-pulsation displacement pump comprises two series-connected cylinders, which are controlled by valves so that the delivery takes place only in one direction. The pistons of the cylinders are controlled through cams which are driven jointly at a constant velocity.

Reciprocating pump: The piston expels liquid through a one-way valve (check valve). The pumping rate is usually adjusted by controlling the distance the piston retracts, thus limiting the amount of liquid pushed out by each stroke, or by the cam rotating speed.

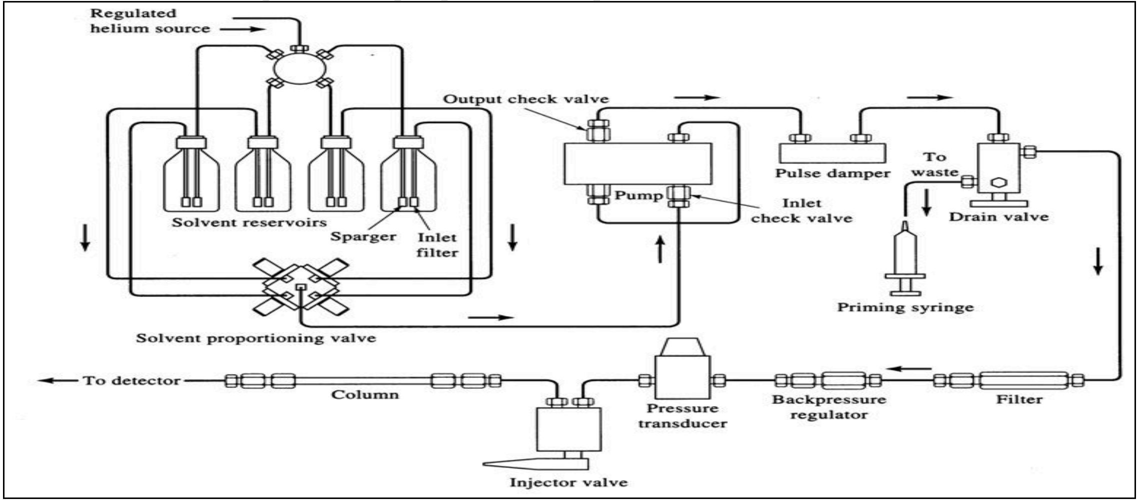


Fig. 3: Instrumentation for High-Performance Liquid Chromatography [3]

Injection device: Injectors for liquid chromatographic systems should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi). They should also produce minimum band broadening and minimize possible flow disturbances. Generally, the most useful and widely used sampling device for modern LC is the microsampling injector valve. Because of their superior characteristics, valves are now used almost to the exclusion of syringe injection. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures.

1.5 DRUG PROFILE

1.5.1 Etodolac [39]

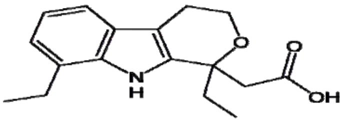


Figure .4: Structure of Etodolac

Chemical name: (*RS*)-2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b] indol-1-yl)acetic acid

Molecular formula: $C_{17}H_{21}NO_3$.

Molecular weight: 287.35 gm/mol,

Appearance: White to slightly yellowish crystals or crystalline powder.

Solubility: Soluble in methanol & nearly insoluble in water

Category: Etodolac is an anti-inflammatory agent with analgesic and antipyretic properties.

Dose: Oral Osteoarthritis

Adult: 600-1000 mg/day in divided doses adjusted according to response.

Max Dosage: 1200 mg daily. Oral Rheumatoid arthritis

Adult: 600-1000 mg/day in divided doses adjusted according to response.

Max Dosage: 1200 mg daily. Oral Acute pain.

Identification: The light absorption in the range 230 nm to 360 nm of a 0.01% w/v solution in methanol exhibits maxima at about 302 nm.

Storage: Store in a cool, dry and dark place.

Pharmacology: Decreases inflammation, pain, and fever, probably through inhibition of cyclooxygenase activity and prostaglandin synthesis Etodolac is an NSAID. Exactly how it works is not known. It may block certain substances in the body that are linked to inflammation. NSAIDs treat the symptoms of pain and inflammation. They do not treat the disease that causes those symptoms. Etodolac is an anti-inflammatory agent with analgesic and antipyretic properties. It is used to treat osteoarthritis, rheumatoid arthritis and control acute pain. The therapeutic effects of Etodolac are achieved via inhibition of the synthesis of prostaglandins involved in fever, pain, swelling and inflammation. Etodolac is administered as a racemate. As with other NSAIDs, the S-form has been shown to be active while the R-form is inactive. Both enantiomers are stable and there is no evidence of R- to S- conversion *in vivo*.

Adverse effects: Etodolac is generally well tolerated and side effects usually are transient reported side effects includes; 1) Headache, 2) nausea, 3) dizziness. 4) Diarrhoea, 5) vomiting, 6) abdominal pain. 7) Joint pain, 8) arthritis, 9) vaginitis 10) skin rash.

Uses: An anti-inflammatory agent with analgesic and antipyretic properties is used to treat osteoarthritis and control acute pain.

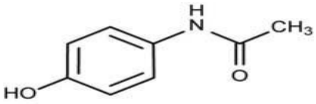


Figure 5: Structure of Paracetamol

1.5.2. Paracetamol [4]

Chemical name: N-(4-hydroxyphenyl) acetanilide, **Molecular formula:** $C_8H_9NO_2$.

Molecular weight: 151.17g/mol,

Category: Paracetamol is used as an **Analgesic and Antipyretic**. **Appearance:** A white or almost white crystalline powder.

Solubility: Paracetamol is soluble in methanol.

Dose: Initial dose of Paracetamol is 250 mg per day.

Pharmacology: Paracetamol exhibit analgesic action by peripheral blockage of pain impulse generation .It produces antipyresis by inhibiting the hypothalamic heat regulating centre. It is weak anti-inflammamatory activity is related to inhibition ofprostaglandin synthesis in the central nervous system.

Pharmacokinetics: Paracetamol (acetaminophen) is generally considered to be a weak inhibitor of the synthesis of prostaglandins (PGs). However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors. Paracetamol also decreases PG concentrations in vivo, but, unlike the selective COX-2 inhibitors, paracetamol does not suppress the inflammation of rheumatoid arthritis. It does, however, decrease swelling after oral surgery in humans and suppresses inflammation in rats and mice. Paracetamol is a weak inhibitor of PG synthesis of COX-1 and COX-2 in broken cell systems, but, by contrast, therapeutic concentrations of paracetamol inhibit PG synthesis in intact cells in vitro when the levels of the substrate arachidonic acid are low (less than about 5 mol/L).

Adverse Effects: Very common: More than 1 in 10 people who take Paracetamol

Common: More than 1 in 100 people who take Paracetamol.

Confusion, Constipation, Diarrhoea, Dry mouth, Euphoria, feeling anxious, feeling nervous, Flatulence, Headaches, Indigestion, itching, Mood changes, Sleeping problems, Stomach pain, sweating, trembling, Vomiting.

2. Methodology

2.1 Determination of λmax of Etodolac:

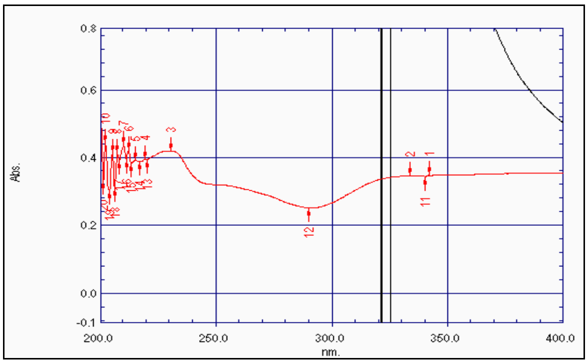


Figure 6: UV spectra of Etodolac

The standard solution of Etodolac was scanned at different concentrations in the range of 200-400 nm and the λmax was found to be 302 nm against reagent blank.

2.2 Determination of λ max of Paracetamol:

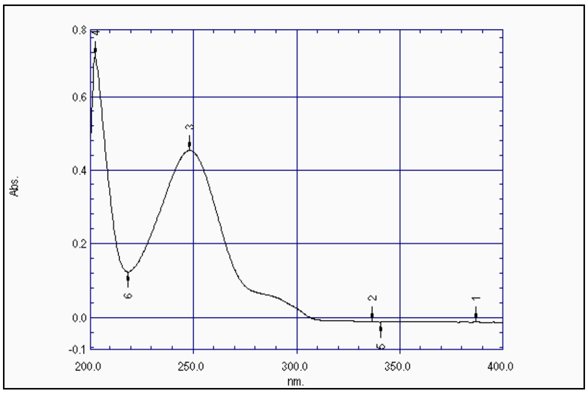


Figure 7: UV spectra of Paracetamol

The standard solution of Paracetamol was scanned at different concentrations in the range of 200-400 nm and the λ max was found to be 240 nm against reagent blank

RP-HPLC Method for Etodolac and Paracetamol.

Determination of Etodolac and Paracetamol in combined tablet dosage form.

METHOD

Solubility of drugs in different solvents: Solubility of both drugs was observed by dissolving them in different solvents.

Table 1: Solubility of drugs in different solvents:

Solvents	Solubility	
	Etodolac	Paracetamol
ACN	+	+
Water	+	++
Methanol	+++	+++
Methylene	+	+

(+++) Symbolizes highly soluble, (++) Symbolizes freely soluble, (+) Symbolizes soluble.

Method:

A. Selection of analytical wavelength: By appropriate dilution of each standard stock solution with methanol, various concentrations of Etodolac and Paracetamol were prepared separately. Each solution was scanned in between the range of 200 nm to 400 nm and their spectra were overlaid. The wavelength selected for the analysis was 235 nm which both the drugs showed significant absorbance. The overlain UV spectra of Etodolac and Paracetamol in the methanol are as shown in figure below.

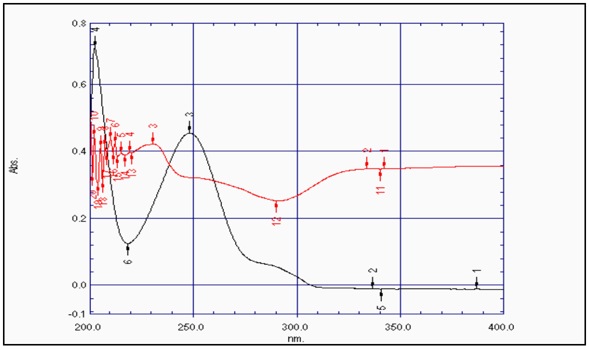


Figure 8: Overlain spectra of Etodolac and Paracetamol

B. Calibration of Etodolac & Paracetamol: Take 10 mg of Etodolac & 10 mg of Paracetamol separately in 100 mL volumetric flask which was then dissolved in 40 mL mobile phase with shaking. Then it was sonicated for 15 min and the volume was made up to 100 mL. Suitable dilutions are made from the standard stock solutions. The linearity of the relationship between peak area and concentration was determined by analyzing six working standards over the concentration range of 10, 20, 30, 40, 50 and 100µg/mL for Etodolac and 10, 20, 30, 40, 50 and 100µg/mL for Paracetamol. Hence the peak areas were recorded against concentration and graph of concentration Vs peak area was plotted.

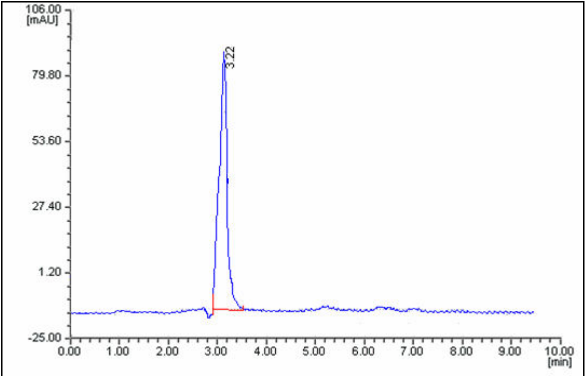


Figure 9: Chromatogram of 10 µg/mL of Etodolac

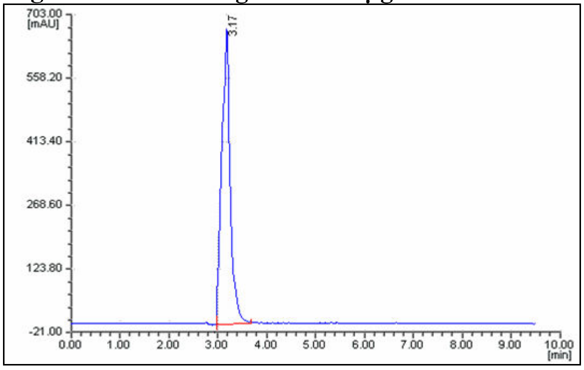


Figure 10: Chromatogram of 100 µg/mL of Etodolac

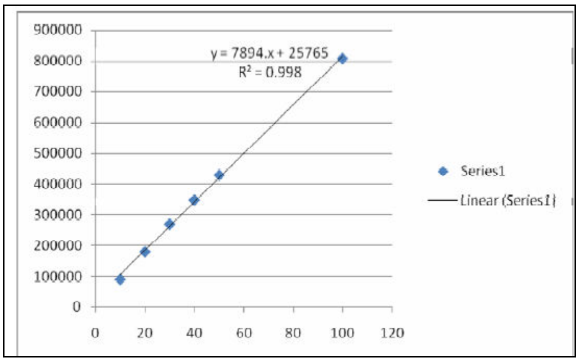


Figure 11 : Calibration curve of Etodolac

Chromatogram of Paracetamol;

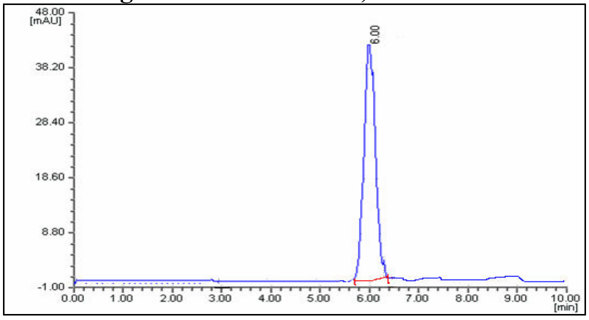


Figure 12: Chromatogram of 10 µg/mL of Paracetamol

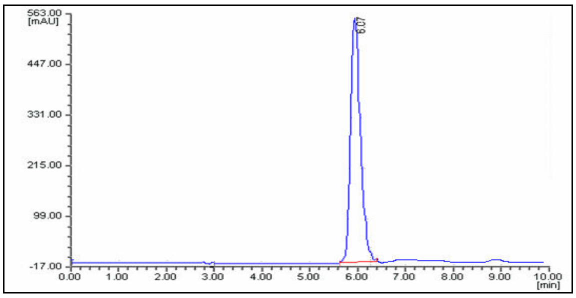


Figure 13: Chromatogram of 100 µg/mL of Paracetamol

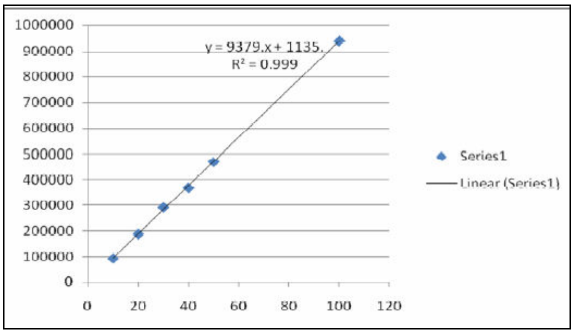


Figure 14: Calibration curve of Paracetamol

Table 2: Calibration of Etodolac and Paracetamol

	Conc (µg/ml)		Area (µv.sec)	
	Etodolac	Paracetamol	Etodolac	Paracetamol
1	10	10	93498.0	91746.0
2	20	20	188453.5	181902.1
3	30	30	291273.8	245698.7
4	40	40	368876.9	348948.0
5	50	50	469843.8	419603.4
6	100	100	939803.8	8473311.8

C. Selection of mobile system: The pure drug of Etodolac and Paracetamol were injected into HPLC system by using different solvent systems. Different mobile phases like acetonitrile: methanol: water, acetonitrile: methanol: potassium dihydrogen phosphate buffer, were tried in order to find the optimum conditions for the separation of Etodolac and Paracetamol. Finally the optimum composition of the mobile phase was selected as methanol (60 mL) mixed with 25 mM phosphate buffer (40 mL), whose pH was adjusted to 6.1 by orthophosphoric acid which gave satisfactory results with sharp well defined and well resolved peaks and acceptable peak parameters as compared to other mobile phases.

D. Preparation of mobile phase: Methanol (60 mL) was mixed with 25 mM phosphate buffer (40 mL), and then pH was adjusted to 6.1 by addition of ortho-phosphoric acid.

E. Degassing the mobile phase: The mobile phase prepared was degassed by ultrasonication for about 15 min, so as to avoid the disturbances caused by dissolved gases.

F. Filtration of mobile phase: The mobile phase after degassing was filtered through 0.45 µm membrane nylon filter to remove the smaller particles that may be present in the mobile phase and which may cause clogging of column.

G. Preparation of standard stock solutions: 10 mg of Etodolac and 10 mg of Paracetamol were weighed separately and dissolved in 40 mL of mobile phase with shaking. Then it was sonicated and the volume was made upto 100 mL by using mobile phase. From standard stock solution of drug, appropriate dilutions were made using the mobile phase and the sample was filtered through 0.2 µm of clear filtrate were injected into the HPLC column.

H. Loading of mobile phase: Filtered and degassed mobile phase was loaded in the reservoir. Priming was done for each freshly prepared mobile phase.

I. Baseline stabilization: The detector was turned on for an hour before the actual run in order to obtain the stable UV light. The mobile phase run was started at desired flow rate and the run was continued until the stable baseline was obtained.

J. Loading of samples: Well prepared and filtered samples of Etodolac and Paracetamol were loaded into the Rheodyne injector port using a syringe and the sample was then injected.

K. Washing the column: Once the analysis of samples was finished, the column was first washed by flushing with the mobile phase for half an hour, afterwards with double distilled water and methanol in 1:1 proportion for another one hour.

L. Selection and Optimization of HPLC method: After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity and accuracy.

M. Chromatogram of working standard for Etodolac and Paracetamol

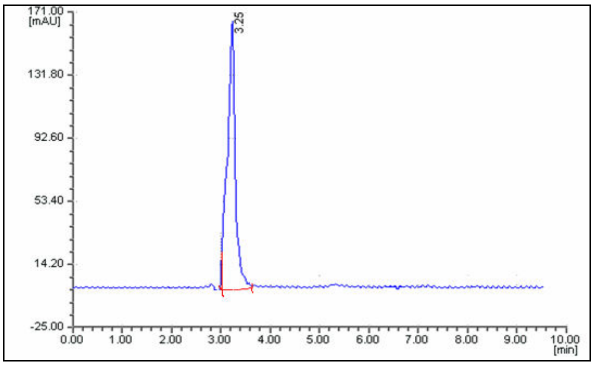


Figure 15: Chromatogram of working standard of 20 µg/ml of Etodolac

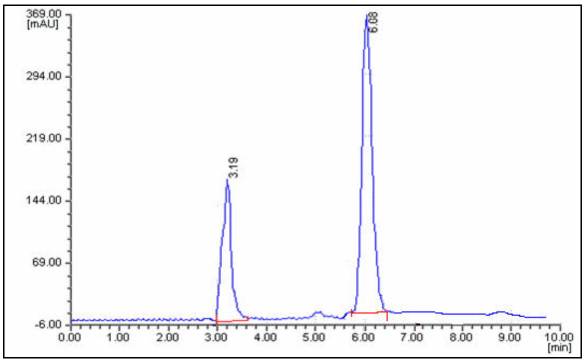


Figure 16: Chromatogram of working standard of 20µg/ml of Etodolac and 50µg/ml of Paracetamol

Table. 3 : Details of chromatogram of working standard containing Etodolac and Paracetamol.

Sr. No	Name	RT (min)	Area (µv.sec)	Theoritical plates	Reaolution	Asymmetry
1	Etodolac	3.19	164729.6	2508.36	2.53	1.51
2	paracetamol	6.09	424509.4	3703.55	2.43	1.63

N. Analysis of Tablet formulation:

Brand: ETOVA-P, **Manufacturer:** Intas Pharmaceuticals

Each Tablet contains: Etodolac 200mg, Paracetamol 500 mg

Procedure: Take twenty tablets, each containing 200 mg of Etodolac and Paracetamol 500 mg. The tablets were crushed to fine powder and amount of powder equivalent 100 mg of Etodolac and 250 mg Paracetamol were weighed and transferred to100 mL dried volumetric flask. Sufficient amount of mobile phase was added to dissolve the content and shaken for 15 min. The volume was made up to 100 mL with mobile phase. Then solution was filtered by using membrane filter and degassed. From this solution appropriate dilutions of Etodolac and Paracetamol were made to get the final concentrations and injected into the system to get the chromatogram. The chromatogram obtained is shown in Figure 18. And the area obtained in each chromatogram of five replicate was correlated with regression equation and the amount found is calculated which was within the limit of label claim as mentioned in Table 3.

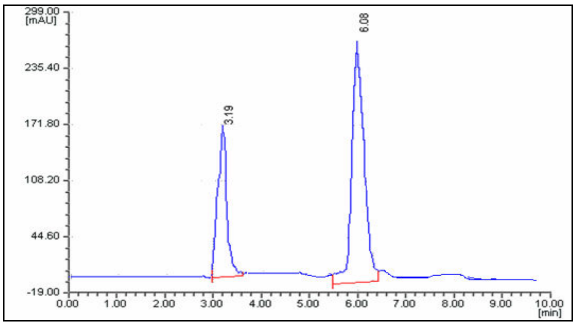


Figure 17: Chromatogram of Etodolac (20 µg/mL) and Paracetamol (50 µg/mL) in Tablet Formulation.

Table. 4: Details of chromatogram of (20 µg/mL) Etodolac and (50 µg/mL) Paracetamol Tablet.

Sr. No	Name	RT(min)	Area (µv.sec)	Theoretical plates	Resolution	Asymmetry
1	Etodolac	3.192	184729.6	2708.36	2.53	1.31
2	paracetamol	6.092	424509.4	3003.55	2.43	1.53

Table 5: Analysis of ETOVA-P Tablet.

Sl. No	Amount present (µg/ml)		Amount found (µg)		% Label claim	
	Etodolac	Paracetamol	Etodolac	Paracetamol	Etodolac	Paracetamol
1	20	50	19.56	49.54	99.90	99.76
2	20	50	19.74	49.58	99.45	99.56
3	20	50	19.44	49.87	99.65	99.81
4	20	50	19.43	49.65	99.48	99.72
5	20	50	19.52	49.58	99.51	99.56

O. Evaluation of analytical method (Method validation):

Linearity and sensitivity: Suitable dilutions of different concentrations using mobile phase were made from the standard stock solutions. The linearity of the relationship between peak area and concentration was determined by analyzing six working standards over the concentration range 10-100 µg/ml for Etodolac and 10-100 µg/ml for Paracetamol. Here the peak areas were recorded against concentration and graph of concentration Vs peak area was plotted. Results obtained are shown in Table 6.

Table 6: Linearity of Etodolac. (n=3)

Standard conc.	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml
Replicates	Peak area					
1	95574.0	188250.4	291073.8	370980.6	470809.3	940467.7
2	93573.1	189609.1	291081.4	366817.3	474861.9	941178.3
3	94895.6	188151.7	291426.4	368709.9	476574.1	945799.6
Mean	94680.9	188670.4	291193.9	368835.9	474081.8	942481.9
± SD	1017.581	814.431	201.415	2084.510	2960.521	2895.126
% RSD	1.0747	0.4316	0.0691	0.5651	0.6244	0.3071

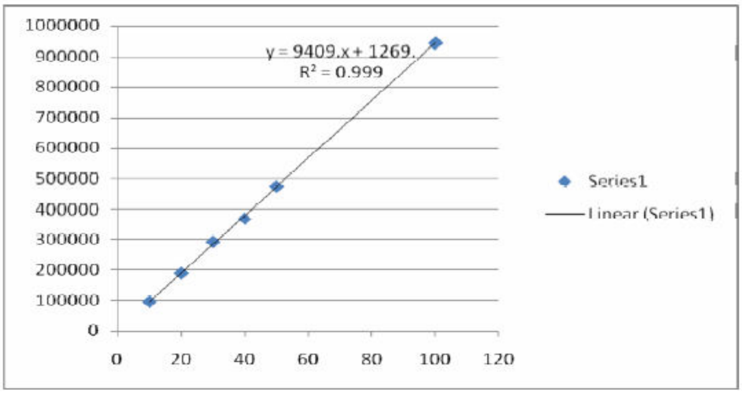


Figure 18: Calibration plot of Etodolac

Table 7: Linearity Paracetamol. (n=3)

Standard conc.	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml
Replicates	Peak area					
1	91574.0	178250.4	261073.8	340980.6	440809.3	870467.7
2	92573.1	179609.1	251081.4	346817.3	424861.9	841178.3
3	91895.6	178151.7	251426.4	348709.9	426574.1	845799.6
Mean	91680.9	178670.4	251193.9	348835.9	424081.8	842481.9
± SD	317.581	414.431	5301.415	5084.510	2260.521	2395.126
% RSD	0.3747	0.2316	1.8691	1.4651	0.6244	0.3071

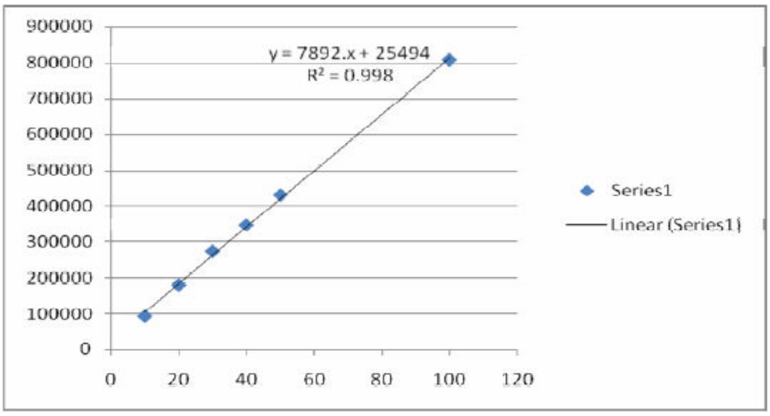


Figure 19: Calibration of Paracetamol

Precision: Repeatability of method was established by analyzing various replicates standards of Etodolac and Paracetamol. All the solutions were analyzed thrice, in order to record any intra-day and inter-day variation in the result. The result obtained for intraday variations are shown in Table 8 and Table 9. And the result obtained for inter-day variations are shown in the Table 10 and Table 11.

Table 8: Intra-day variability of Etodolac

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV.sec)	±SD	% RSD
	Trial 1	Trial 1	Trial 1			
20	182980.4	187634.8	185643.9	187654.7	3706.217	1.9868
30	294396.5	295396.5	298396.5	294696.5	294696.5	0.6868
40	371238.2	379238.2	371838.2	371438.2	745.845	0.2101

Table 9: Intra-day variability of Paracetamol

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV.sec)	±SD	% RSD
	Trial 1	Trial 1	Trial 1			
20	182180.4	183634.8	185143.9	187654.7	406.217	0.9868
30	264396.5	275396.5	258396.5	264696.5	2410.5	0.6868
40	341238.2	359238.2	351838.2	341438.2	2745.845	0.86

Table 10: Inter-day variability of Etodolac

Conc. (µg/ml)	Peak area (µV.sec)			Mean area(µV.sec)	±SD	% RSD
	Day 1	Day2	Day3			
20	182240.4	187104.8	182343.9	183054.7	3711.217	1.6868
30	294396.5	295396.5	298396.5	294696.5	1696.5104s	0.6868
40	371238.2	379238.2	371838.2	371438.2	545.845	0.2117

Table 11: Inter-day variability of Paracetamol

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV.sec)	±SD	% RSD
	Day 1	Day2	Day3			
20	**182740.4**	**187464.8**	**186343.9**	**183054.7**	**3711.217**	**0.1368**
30	**254396.5**	**265396.5**	**268396.5**	**264696.5**	**3696.510**	**0.368**
40	**371238.2**	**379238.2**	**371838.2**	**371438.2**	**545.845**	**2.117**

Accuracy: To check the accuracy of proposed method, level of recovery carried out at 80, 100 and 120% of the concentration as per standard addition method. Here the product was accessed by addition of series of known amount of standard drug in the product and then the contents were estimated by assay method. The % recovery for Etodolac and Paracetamol is in the range. Results are shown in Table 12.

Table.12: Recovery studies of Etodolac and Paracetamol.

Tablet Sample	Level of recovery %	Amount taken (µg/ml)		Amount of standard added (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
	Drug	ET	PCM	ET	PCM	ET	PCM	ET	PCM
ETOVA-P	80	20	50	16	40	35.56	89.34	99.34	99.64
	80	20	50	16	40	35.45	89.56	99.43	99.53
	80	20	50	16	40	35.76	89.67	99.65	99.75
	100	20	50	20	50	39.57	99.23	99.32	99.42
	100	20	50	20	50	39.68	99.49	99.53	99.63
	100	20	50	20	50	39.81	99.51	99.38	99.48
	120	20	50	24	60	43.42	108.66	99.56	99.66
	120	20	50	24	60	43.56	108.45	99.89	99.99
	120	20	50	24	60	43.78	108.76	99.44	99.54

Table.13: Statistical Validation of ETOVA- P 200 Tablet

Level of % recovery	% Mean		±SD		%RSD	
	ET	PCM	ET	PCM	ET	PCM
80	99.75	99.82	0.1852	0.1234	0.1955	0.1126
100	99.54	99.74	0.3771	0.1156	0.3788	0.1158
120	99.28	99.85	0.4265	0.0987	0.4317	0.0972

Limit of detection (LOD): LOD is calculated from the formula

$LOD = 3.3\sigma/S$, σ= Standard deviation of the response, s = slope of the calibration curve

Etodolac: 0.10123 µg/mL, Paracetamol: 0.09861 µg/mL

Limit of Quantitation (LOQ): LOQ is calculated from the formula $LOQ=10\sigma/S$

σ= Standard deviation of the response S= slope of the calibration curve

Etodolac: 0.2193 µg/mL, Paracetamol: 0.19509 µg/mL

Linearity Range: The range shown by is given as follows

Etodolac: 10-100 µg/mL, Paracetamol: 10-100 µg/mL

Robustness: Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and pH was altered. Variation of mobile phase pH and ratio are seemed to have greater impact on resolution and hence it should be meticulously controlled.

Analysis of tablet formulation: After analysis of all the different brands of tablets the amount found is calculated which was within the limit of label claim.

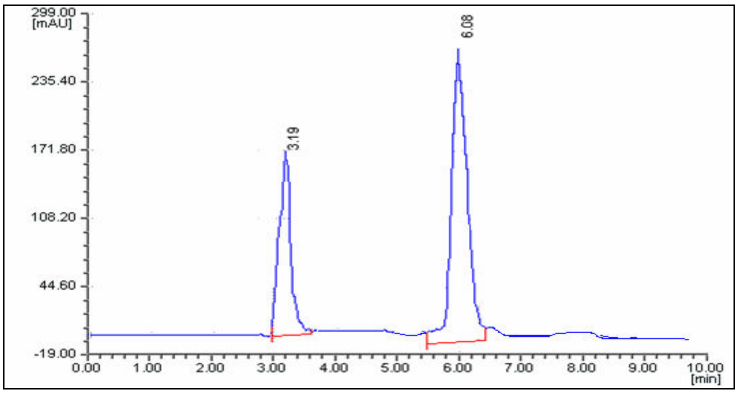


Figure 20: Chromatogram of Etodolac (20 µg/mL) and Paracetamol (50 µg/mL tablet) in Tablet Formulation.

Table 14: Analysis of tablet

Sr. No.	**Amount present in (µg)**		**Amount found in (µg)**		**% Label claim**	
	ET	PCM	ET	PCM	ET	PCM
1	20	50	19.95	49.78	99.92	99.82
2	20	50	19.90	49.87	99.45	99.35
3	20	50	19.89	49.67	99.83	99.73
4	20	50	19.97	49.54	99.56	99.46
5	20	50	19.87	49.83	99.89	99.79

3. Result And Discussion

3.1 HPLC Method: RP-HPLC method was developed and validated for estimation of Etodolac and Paracetamol from bulk drug and tablet formulation. Method was validated by using different parameters likes linearity, precision, accuracy, and limit of detection, limit of quantitation, specificity, range and robustness. Method of RP High performance liquid chromatography has been successfully employed for simultaneous estimation of Etodolac and Paracetamol from bulk and tablet dosage form. Method was validated as per ICH and USP guidelines by using different parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, specificity, range and robustness. The linearity of Etodolac was observed in the range of 10-100 µg/mL and that of Paracetamol was in the range of 10-100 µg/mL. Detection wavelength used was 235 nm with correlation coefficient 0.999 and 0.998 for Etodolac and Paracetamol respectively. The limit of detection was found to be 0.10123 µg/mL and 0.09861 µg/mL for Etodolac and Paracetamol respectively and limit of quatitation 0.2193 µg/mL and 0.19509 µg/mL for Etodolac and Paracetamol respectively. The percent relative standard deviation and high percent recovery data were satisfactory and confirms accuracy, precision and reliability of the method. Thus the methods are simple, accurate, precise, economical and reproducible for routine estimation of Etodolac and Paracetamol respectively from bulk drug and formulations.

4. Conclusion

The proposed method was found to be Simple, Accurate, Precise, economical, Reproducible.

The sample assay results and recoveries in tablets were in good agreement with their respective label claims and added standard sample, and thus, suggested noninterference of formulation excipients in the estimation. Hence the propose method can be used for the routine quality control analysis of Etodolac and Paracetamol in bulk drug as well as tablet formulations.

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