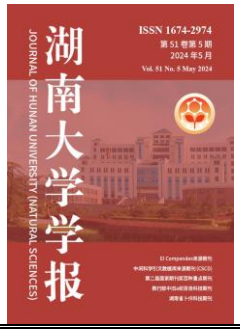

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Development and Validation of a Method for Analyzing the Combination of Etoricoxib and Acetaminophen in Tablet Dosage Form

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Abstract: This study aimed to create a tablet with etoricoxib and acetaminophen to improve patient compliance. A selective, simple, precise, and economical HPLC method was developed and validated for quantifying acetaminophen and etoricoxib in tablet form. The drugs were separated using a mobile phase consisting of methanol, acetonitrile, and phosphate buffer at pH 3.4, flowing through an ODS C18 column at a rate of 1.0 ml/min at 25°C. At concentrations of 10-180 µg/ml for acetaminophen and 1-50 µg/ml for etoricoxib, linearity was established with a regression coefficient of 0.9992. No interaction or degradation was found for the combination product. ICH-compliant results with a 2% RSD were satisfactory. This method is robust to pH, temperature, detector wavelength, column, and mobile phase composition changes. This method is ideal for routine analysis of acetaminophen and etoricoxib tablets because of its simplicity and low reagent cost. The HPLC method for estimating acetaminophen and etoricoxib in combined dosage forms was sensitive, accurate, precise, linear, stable, and robust. Acetaminophen and etoricoxib in pharmaceutical formulations can be tested using the proposed method for quality control and routine analysis. Our combination formulation method yielded a non-friable tablet with good performance, hardness, and disintegration. We recommend this HPLC method for the routine analysis of raw materials and formulations.

Keywords: acetaminophen, etoricoxib, HPLC, validation, ICH guidelines.

片剂中依托昔布和对乙酰氨基酚组合的分析方法的开发和验证

摘要：本研究旨在制造一种含有依托昔布和对乙酰氨基酚的药片，以提高患者的依从性。开发并验证了一种选择性强、简单、精确且经济的高效液相色谱方法，用于定量片剂形式的对乙酰氨基酚和依托昔布。使用由甲醇、乙腈和pH 3.4磷酸盐缓冲液组成的流动相分离药物，以1.0毫升/分钟的速率在25摄氏度下流过消耗臭氧层物质C18柱。在对乙酰氨基酚的浓度为10-180微克/毫升和依托昔布的浓度为1-

50微克/毫升时，建立了线性，回归系数为0.9992。组合产品未发现相互作用或降解。符合脑出血的结果令人满意，相对标准偏差为2%。该方法不受pH、温度、检测器波长、色谱柱和流动相成分变化的影响。该方法简单易行，试剂成本低，是常规分析对乙酰氨基酚和依托昔布片剂的理想选择。用于评估复方制剂中对乙酰氨基酚和依托昔布的高效液相色谱方法灵敏、准确、精确、线性、稳定且耐用。可以使用建议的方法对药物制剂中的对乙酰氨基酚和依托昔布进行质量控制和常规分析。我们的复方制剂方法生产出了一种性能、硬度和崩解性良好的非易碎药片。我们推荐使用这种高效液相色谱方法对原材料和制剂进行常规分析。

关键词：对乙酰氨基酚、依托昔布、高效液相色谱、验证、脑出血指南。

1. Introduction

Combination medications are defined as fixed-dose combinations (FDCs), which are two or more drugs (marketed or unmarketed) mixed in a single pill or two or more distinct drugs packaged together [1–3]. FDCs have been commercially marketed for many years, but several regulatory agencies and the World Health Organization have recently established nonclinical development guidelines. There are currently no ICH guidelines for combination drugs [4]. Compared with monotherapy, combining medications to improve clinical outcomes has higher efficacy. It can reduce the risk of adverse effects by using 17 lower individual dosages [2, 5].

It is a routine therapeutic practice to combine NSAIDS and acetaminophen because of their synergistic analgesic effectiveness. Both drugs work in distinct ways [6]. The addition of acetaminophen enhances the antipyretic activity. Because COX-2 inhibitors in fixed dosage combinations with acetaminophen are only used to treat acute pain in the short term, their safety would not be a problem [7]. Both acetaminophen and an NSAID have been investigated and found to be effective pain relievers [8]. Combining two analgesics with potentially distinct mechanisms of action may be more effective than each medication alone, minimizing the need for further analgesics after surgery [9, 10].

Etoricoxib is a selective COX-2 inhibitor. It reveals that dosage-dependent inhibition of COX-2, without inhibition of COX-1, does not decrease gastric prostaglandin production and has no effect on platelet function over the therapeutic dose range [11].

Etoricoxib is administered at a daily dosage of 60 mg for osteoarthritis and chronic musculoskeletal pain, 60 and 120 mg for primary dysmenorrhea, 90 mg for rheumatoid arthritis, and 120 mg for acute gouty arthritis and acute pain following dental surgery [12]. In the United Kingdom, etoricoxib, a once-daily drug for symptomatic relief in treating osteoarthritis, has been approved.

Acetaminophen is the most frequently used analgesic for pain relief [13]. Acetaminophen comes in a variety of forms, including simple (over-the-counter) and more complex (mixed with tramadol) or non-complex (in combination with codeine phosphate, ascorbic acid, or diphenhydramine hydrochloride, as well as NSAIDs such as ibuprofen or propyphenazone). Acetaminophen comes in the form of pills, effervescent tablets, suspension, powder to make oral liquid medicine (sachets), and rectal suppositories. Acetaminophen has a 30-min clinical impact when taken orally. The amount of acetaminophen in oral medications varies; most comprise 500 mg, but certain (mostly complicated) formulations contain 325 mg, 750 mg, or even 1000 mg (e.g., Febrisan, Coldrex, Efferalgan Forte, Codrex MaxGrip, Flucontrol Hot) [14].

This study aimed to create and validate an analytical technique for quantitatively determining acetaminophen and etoricoxib in a tablet formulation and to compare in vitro results with reference dose forms. The chromatographic separations of the two medications were examined by HPLC, and we investigated the dissolving characteristics and melting points using DSC to make additional estimates and measurements of acetaminophen and etoricoxib in tablet format. Furthermore, our research created a unique formulation for the combination of our medications.

2. Method

2.1. Materials and Reagents

We used the following chemicals and reagents. Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from VWR specialty. Merck supplied the water (HPLC grade). LabChem Inc. provided potassium dihydrogen phosphate (AR grade) and phosphoric acid (AR grade). The rest of the reagents were of HPLC grade. The following drugs and film-coated tablets (FCT) were purchased from Dar Al

Dawa Pharmaceuticals: acetaminophen batch number L397sw97272021 and etoricoxib batch number R836bnw4852021.

2.2. Instrumentation

The analysis was performed on an HPLC system, the Thermo Finnigan Surveyor with Item ID TFS-SY-0330. The chromatographic separation was achieved using an ODS C18 column measuring 250 mm x 4.6 mm with a particle size of 5 µm. The system included a UV-VIS Plus Detector, a solvent delivery system pump, an LC pump plus, and an Autosampler plus. The software used was Chromoquest 5.0.

2.3. Preparation of the Mobile Phase

Different quantities of mobile phases containing water, acetonitrile, and methanol were tested, along with different buffer concentrations, before determining the optimal mixture of methanol, acetonitrile, and phosphate buffer at a pH of 3.4 (40:20:40 v/v). A 0.45-m membrane filter and sonication were used to filter and degas the mobile phase.

2.4. Preparation of the Standard Solutions

The standard stock solutions of acetaminophen and etoricoxib were prepared by weighing 100 mg of acetaminophen and 18 mg of etoricoxib. These were dissolved in methanol before being transferred to separate 100-ml volumetric flasks. The volume was brought up to 100 ml using the mobile phase. Subsequently, 1 ml of each solution was diluted with 10 ml of diluent to obtain the standard solutions with concentrations of 100 µg/ml for acetaminophen and 18 µg/ml for etoricoxib.

2.5. Sample Solution Preparation

Twenty tablets containing 500 mg of acetaminophen and 90 mg of etoricoxib were weighed and broken into powder. The powder equal to 500-mg acetaminophen and 90-mg etoricoxib was then weighed and transferred to a 100-ml volumetric flask containing 80 ml of the mobile phase. The mixture was sonicated for 20 min. To obtain a concentration of 100-µg/ml acetaminophen and 18-µg/ml etoricoxib, the volume was raised to 100 ml using the mobile phase, and then 1 ml was diluted in 50-ml diluent. A 0.45-µm membrane filter was used to filter the contents.

Different mobile phases containing water, acetonitrile, methanol, and various buffers in varying proportions were tested, and the mobile phase selected was a mixture of methanol, acetonitrile, and phosphate buffer at a pH of 3.4 in a ratio of 40:20:40 (v/v). This combination yielded excellent resolution and peak parameters for both acetaminophen and etoricoxib. According to the ICH recommendations, the technique validation criteria assessed were precision, accuracy, limit of detection, limit of quantitation, linearity, and robustness. The FDA-recommended validation criteria were not exceeded.

2.6. Thermal Degradation Test Preparation

Using differential scanning calorimetry (DSC), we identified the melting temperature for acetaminophen and etoricoxib separately and then placed the melting temperature for each ingredient after mixing them.

2.7. Selectivity Test Preparation

The sample solution was prepared by dissolving the active ingredients in the mobile phase and injecting them with placebo content (reference formulation) into the system.

2.8. Dissolution Test Preparation

The drug dissolution test predicts in vivo drug release profiles by providing information on in vitro drug release. Then, the samples are validated by HPLC to assure that they meet the requirements as per ICH guidelines. The dissolution medium (0.1 HCL in 1000-ml water) is placed in the vessels of the dissolution unit. The tablet is placed within the medium in the six vessels, and the dissolution apparatus is operated. The sample solution is collected from each vessel after 30 min and diluted by the mobile phase to prepare for HPLC analysis.

3. Results

The best chromatographic conditions for measuring acetaminophen and etoricoxib simultaneously were based on each drug's retention time and resolution [15]. It was found that the mixture of the mobile phase gave the best resolution at pH 3.4. The best chromatographic conditions for measuring acetaminophen and etoricoxib simultaneously are shown in Table 1.

Table 1 The best chromatographic conditions for the measurement of acetaminophen and etoricoxib simultaneously (The authors)

Column	C18- column (250 mm x 4.6 mm), 5 µm
Solvent system (mobile phase)	methanol: acetonitrile: phosphate buffer pH 3.4 (40:20:40 v/v)
Flow rate	1.0 ml/min
Detection	242nm wavelength
Injection volume	20 µl
Oven temperature	25 c
Run time	7 minutes
Retention time Paracetamol Etoricoxib	 4 min 6.5 min

3.1. Identification

According to the ICH guidelines, the purity of our compounds was determined using infrared (IR) spectroscopy, which provided us with the IR spectrum graphs for acetaminophen and etoricoxib. Both graphs indicate that we have pure compounds from our active ingredients. Furthermore, the identification of the blank

diluent and our drugs was conducted using HPLC under the previously mentioned chromatographic conditions. The identification of the blank diluent shows no peak, as shown in Fig. 15, which indicates no interference between the peaks of our drugs and any other identified peaks. As shown in Fig. 2 and 3, the peaks of the drugs have good symmetry and resolution.

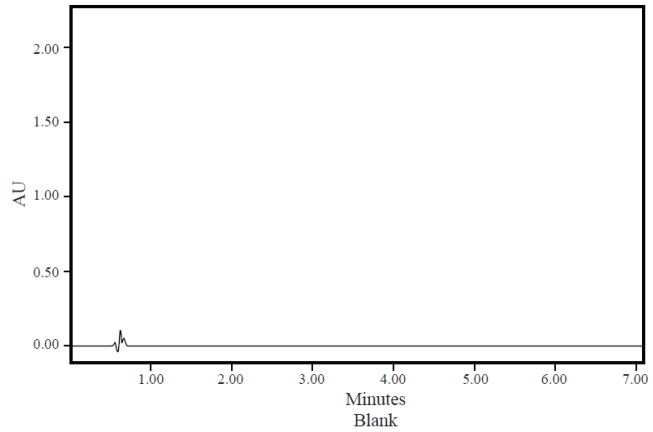

Fig. 1 Chromatogram for a sample containing the blank diluent only under the chromatographic conditions (The authors)

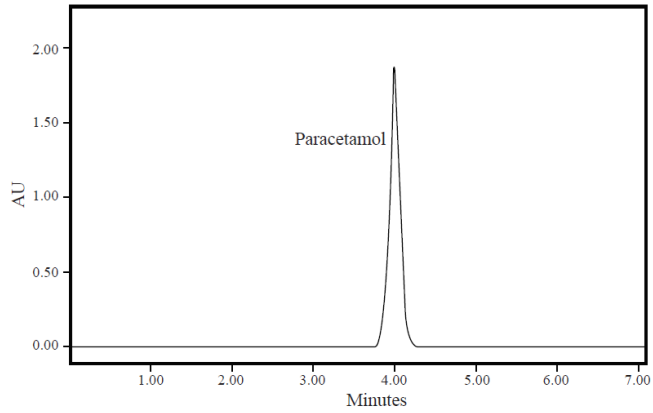

Fig. 2 Chromatogram of a sample containing acetaminophen under the chromatographic conditions (The authors)

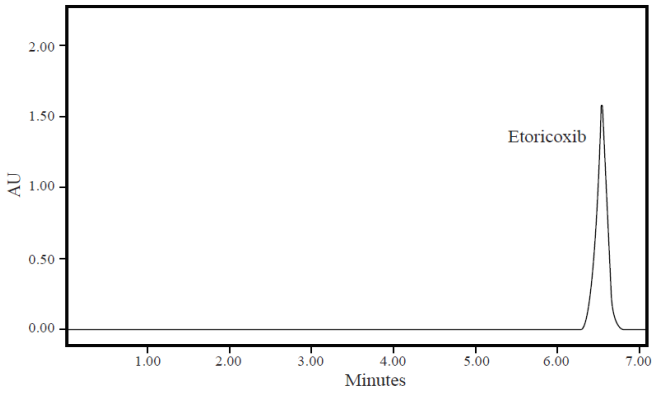

Fig. 3 Chromatogram of a sample containing etoricoxib under the chromatographic conditions (The authors)

3.2. Formulation

The formulation development process involves repeated experimentation with the ingredient substances to obtain the intended characteristics of the final product, e.g., color, taste, effectiveness, performance, or shelf life.

To create a high-performance tablet of our combination, it is essential to incorporate disintegrants, glidants, and lubricants as inactive ingredients. These components play a crucial role in enhancing the tablet's hardness and overall performance. We conducted numerous trials using various ingredients in different amounts to determine the optimal formulation, as outlined in Table 2. The addition of disintegrants is aimed at improving the solubility and dissolution of the tablet. Glidants are included to enhance the flowability and reduce static charge between the particles. Lubricants are added to cover the granules and increase the surface area.

Table 2 Active and inactive ingredient amounts per tablet and 20 tablets (The authors)

Item		**Per tablet (mg)**	**Per 20 tablets (mg)**
Acetaminophen	Active ingredient	500	10000
Etoricoxib	Active ingredient	90	1800
Avicel (101-102)	Super disintegrate	164	3280
Cross povidone	Disintegrate	30	600
Aerosol	Glidant	8	160
Mg stearate	Lubricant	8	160
Total weight		800	16000

All the ingredients were sieved using mesh #36 and mixed in the drum mixer for 2-3 minutes, except Mg stearate, which was sieved using mesh #60 and then mixed for 5 min. Then, compression was applied in a single punch machine (cadmic) to obtain an 800-mg tablet.

As per the ICH guidelines, a disintegration test was performed for six tablets in 2.3 min.

The friability test was performed for 20 tablets; the result was 0.562%, which is acceptable because the normal range must be < 1%.

A hardness test was performed for 10 tablets, and the results are listed in Table 3. All the results are within the accepted range of 800-100 N.

Table 3 Hardness test results (The authors)

Tablet 1	80 N
Tablet 2	82 N
Tablet 3	81 N
Tablet 4	85 N
Tablet 5	83 N
Tablet 6	85 N
Tablet 7	85 N
Tablet 8	75 N
Tablet 9	78 N
Tablet 10	81 N

3.3. System Precision

The purpose of system precision is to evaluate the degree of agreement between individual test results when the procedure is repeated 10 times with the same

homogenous sample [16]. As shown in Tables 4 and 5, according to the ICH criteria, the RSD% readings were less than 2%, showing adequate system precision, while the accuracy was between 98% and 102%. Both are within the acceptable range, indicating that the system technique is accurate.

Table 4 System parameters for etoricoxib for simultaneous measurement of the diluent containing acetaminophen and etoricoxib (The authors)

Etoricoxib					
Injection precision					
Sample ID	**Area**	**Actual Concentration of Etoricoxib (mg)**	**Theoretical Concentration of Etoricoxib (mg)**	**Accuracy**	**RSD**
Inj 1	101671	11.6	11.5	101	0.9
Inj 2	100432	11.5	11.5	100	
Inj 3	102149	11.7	11.5	101	
Inj 4	103050	11.8	11.5	102	
Inj 5	103009	11.8	11.5	102	
Inj 6	103236	11.8	11.5	102	
Inj 7	101828	11.6	11.5	101	
Inj 8	100977	11.5	11.5	100	
Inj 9	101431	11.6	11.5	101	
Inj 10	101293	11.6	11.5	101	

Table 5 System parameters for acetaminophen for simultaneous measurement of the diluent containing acetaminophen and etoricoxib (The authors)

Acetaminophen					
Injection precision					
Sample ID	**Area**	**Actual Concentration of Acetaminophen (mg)**	**Theoretical Concentration of Acetaminophen (mg)**	**Accuracy**	**RSD**
Inj 1	54859	58.3	58	101	1.3
Inj 2	56162	59.8	58	103	
Inj 3	54710	58.1	58	100	
Inj 4	55056	58.5	58	101	
Inj 5	55546	59.1	58	102	
Inj 6	55765	59.3	58	102	
Inj 7	53871	57.2	58	99	
Inj 8	55801	59.4	58	102	
Inj 9	55441	59.0	58	102	
Inj 10	55480	59.0	58	102	

3.4. Method Precision

The method precision was achieved by analyzing a mixture of two drugs six times. The RSD values were below 2%, indicating a precise method for samples in the diluent. In addition, the recovered concentration range was found to be 98-102% for both samples (Tables 6 and 7).

Table 6 Precision of the analytical method for a sample containing acetaminophen (The authors)

Acetaminophen Day 1					
Sample ID	**Acetaminophen Area**	**Theoretical Concentration of Acetaminophen (mg)**	**Actual Concentration of Acetaminophen (mg)**	**Accuracy**	**RSD**
Sample1	92878	100.0	100.7	100.7	0.83
Sample2	91277	100.0	98.9	98.9	
Sample3	93257	100.0	101.1	101.1	
Sample4	92547	100.0	100.3	100.3	
Sample5	93257	100.0	101.1	101.1	
Sample6	92324	100.0	100.0	100.0	

Table 7 Precision of the analytical method for a sample containing etoricoxib (The authors)

Etoricoxib Day 1					
Sample ID	**Etoricoxib Area**	**Theoretical Concentration of Etoricoxib (mg)**	**Actual Concentration of Etoricoxib (mg)**	**Accuracy**	**RSD**
Sample1	159879	18.0	17.8	98.8	0.81
Sample2	160524	18.0	17.9	99.2	
Sample3	162172	18.0	18.0	100.2	
Sample4	163271	18.0	18.1	100.8	
Sample5	160927	18.0	17.9	99.4	
Sample6	162871	18.0	18.1	100.6	

According to the ICH guidelines, the data presented in Tables 6 and 7 are within the accepted limits. The RSD percentage values were below 2%, indicating suitable system precision, and the accuracy percentage

was between 98% and 102% according to ICH guidelines. Therefore, the presented method was found to be precise.

3.5. Intermediate Precision

This was achieved using different equipment and running composite samples for two days. The six sample preparations were evaluated on the first day, and the results (assay % and RSD %) were collected. The analysis was performed on the second day with separate analysts using similar chromatographic settings and the same newly generated concentration. Tables 8 and 9 show that the test results were in the 99%-102% range.

Table 8 Intermediate precision of the analytical method for a sample containing acetaminophen (The authors)

Acetaminophen Day 2					
Sample ID	**Acetaminophen Area**	**Theoretical Concentration of Acetaminophen (mg)**	**Actual Concentration of Acetaminophen (mg)**	**Accuracy**	**RSD**
Sample1	93254	100.0	101.1	101.1	1.26
Sample2	93265	100.0	101.1	101.1	
Sample3	92744	100.0	100.5	100.5	
Sample4	90417	100.0	97.9	97.9	
Sample5	93254	100.0	101.1	101.1	
Sample6	93211	100.0	101.0	101.0	

Table 9 Intermediate precision of the analytical method for a sample containing etoricoxib (The authors)

Etoricoxib Day 2					
Sample ID	**Etoricoxib Area**	**Theoretical Concentration of Etoricoxib (mg)**	**Actual Concentration of Etoricoxib (mg)**	**Accuracy**	**RSD**
Sample1	160544	18.0	17.9	99.2	0.95
Sample2	162478	18.0	18.1	100.4	
Sample3	161472	18.0	18.0	99.8	
Sample4	165265	18.0	18.4	102.0	
Sample5	162447	18.0	18.1	100.3	
Sample6	163287	18.0	18.1	100.8	

The RSD % values and assay obtained are within the accepted range, which indicates a valid method.

3.6. Linearity and Range

The formulation's linearity was tested at six concentration levels ranging from 10 to 180 µg/ml for acetaminophen and 1-50 µg/ml for etoricoxib. Following a triple evaluation of each preparation, a linear regression analysis of the average peak areas versus the concentration of the investigated levels was undertaken [15].

The equation for the regression line was y = 897.64x + 2522.2 (R^2 = 0.999) for acetaminophen and y = 9574.7x – 8835.2 (R^2 = 0.999) for etoricoxib. The results show an excellent correlation between the response factor and drug concentration within the indicated concentration range.

The calibration curve results can be found in Fig. 4 and 5 and Tables 10 and 11.

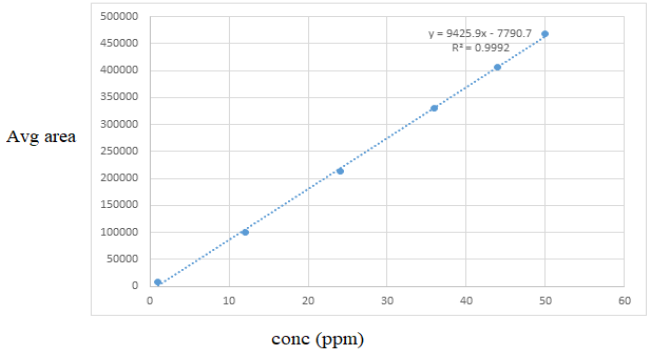

Fig. 4 Calibration curve for etoricoxib (The authors)

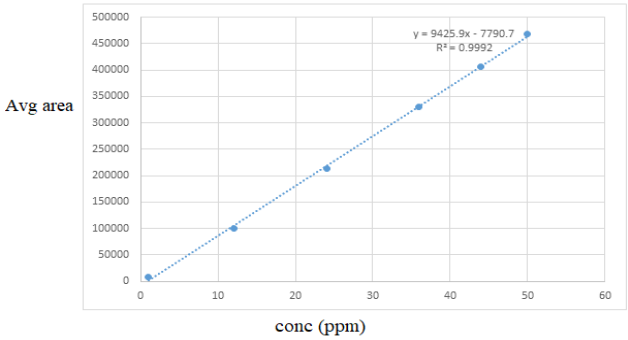

Fig. 5 Calibration curve for acetaminophen (The authors)

Table 10 RSD % for each calibration curve of etoricoxib (The authors)

#	**ppm**	**Etoricoxib Area**	**Average**	**RSD**
Cal1 rep1	1	8547	8460	1.08
Cal1 rep2		8365		
Cal1 rep3		8468		
Cal2 rep1	12	100257	100327	0.07
Cal2 rep2		100396		
Cal2 rep3		100329		
Cal3 rep1	24	221473	214160	5.19
Cal3 rep2		219637		
Cal3 rep3		201369		
Cal4 rep1	36	331478	329713	1.19
Cal4 rep2		325207		
Cal4 rep3		332454		
Cal5 rep1	44	401786	405595	1.44
Cal5 rep2		402680		
Cal5 rep3		412320		
Cal6 rep3	50	462547	469118	1.21
Cal6 rep3		472554		
Cal6 rep3		472254		

Etoricoxib's calibration curve was linear across the concentration range examined, as illustrated in Fig. 4. The correlation value was 0.999, showing strong linearity for etoricoxib within the stated concentration range that follows the validation method's linearity. Acetaminophen was studied similarly, and the results (AUCs and RSD %) are shown in the table below.

Table 11 RSD % for each calibration curve of acetaminophen (The authors)

#	ppm	Acetaminophen Area	Average	RSD
Cal1 rep1	10	9814	9902	0.78
Cal1 rep2		9931		
Cal1 rep3		9961		
Cal2 rep1	20	19670	19718	0.23
Cal2 rep2		19725		
Cal2 rep3		19759		
Cal3 rep1	40	40235	39680	1.25
Cal3 rep2		39527		
Cal3 rep3		39278		
Cal4 rep1	80	77547	77325	0.49
Cal4 rep2		77544		
Cal4 rep3		76884		
Cal5 rep1	140	125472	126142	0.61
Cal5 rep2		125967		
Cal5 rep3		126987		
Cal6 rep3	180	164782	164256	0.81
Cal6 rep3		162739		
Cal6 rep3		165247		

A good linear relationship (R^2 = 0.999) was observed between the concentrations and the average area. The calibration curve of acetaminophen was linear over the concentration range studied, as shown in Fig. 5. The correlation coefficient was 0.999, which indicates good linearity for acetaminophen within the given concentration range, which is consistent with the linearity of the validation method.

The calibration curves were repeated for each drug within the same concentration range on another day. The results revealed linearity in the drug range, and the correlation coefficient was 0.999. Moreover, the RSD % results were within the acceptable limits.

3.7. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is calculated using the formula LOD = 3.3(Sy/S) based on the response standard deviation (Sy) of the curve and the slope of the calibration curve (S). The response standard deviation is determined using the standard deviation of the y-intercepts of the regression lines.

The calculation technique, as previously stated, is based on the standard deviation of the response (SD) and the slope of the calibration curve (S) using the formula LOQ = 10(Sy/S).

Table 12 shows the LOD and LOQ results for acetaminophen and etoricoxib. They were calculated on the basis of the calibration curves for each drug.

Table 12 LOD and LOQ for acetaminophen and etoricoxib (The authors)

	Etoricoxib (µg/ml)	Acetaminophen (µg/ml)
LOD	0.243114	0.184562
LOQ	0.73671	5.592786

3.8. Recovery Accuracy

Samples at three distinct concentrations, 70%, 100%, and 130%, were tested to determine accuracy. The injection was conducted in triplicate at each concentration level compared with the reference sample. Tables 13 and 14 provide the findings. The recovery equation is % accuracy = (actual concentration/theoretical concentration) x 100. According to the ICH criteria, the permitted limits of recovery are 98%-102%.

Table 13 Accuracy determination for etoricoxib (The authors)

Etoricoxib Recovery						
Sample ID	**Etoricoxib Area**	**Actual Concentration (mg)**	**Theoretical Concentration (mg)**	**Accuracy**	**Average Accuracy**	**RSD %**
70% recovery of etoricoxib Sample1	110142	12.5	12.6	99	98.7	0.7
70% recovery of etoricoxib Sample2	109547	12.4	12.6	99		
70% recovery of etoricoxib Sample3	108458	12.3	12.6	98		
100% recovery of etoricoxib Sample1	162748	18.1	18.2	100	99.7	0.6
100% recovery of etoricoxib Sample2	160542	17.9	18.0	99		
100% recovery of etoricoxib Sample3	162440	18.1	18.0	100		
130% recovery of etoricoxib Sample1	210001	23.1	23.4	99	99.3	0.5
130% recovery of etoricoxib Sample2	211741	23.3	23.4	100		
130% recovery of etoricoxib Sample3	212212	23.3	23.4	100		

Table 14 Accuracy determination for acetaminophen (The authors)

Acetaminophen Recovery						
Sample ID	**Acetaminophen Area**	**Actual Concentration (mg)**	**Theoretical Concentration (mg)**	**Accuracy**	**Average accuracy**	**RSD**
70% recovery of acetaminophen Sample1	65210	69.8	70.0	100	100.89	1.0
70% recovery of acetaminophen Sample2	66247	71.0	70.0	101		
70% recovery of acetaminophen Sample3	66285	71.0	70.0	101		
100% recovery of acetaminophen Sample1	92475	100.2	100.0	100	100.44	0.6
100% recovery of acetaminophen Sample2	92321	100.0	100.0	100		
100% recovery of acetaminophen Sample3	93254	101.1	100.0	101		
130% recovery of acetaminophen Sample1	120587	131.5	130.0	101	101.20	0.9
130% recovery of acetaminophen Sample2	119524	130.3	130.0	100		
130% recovery of acetaminophen Sample3	121740	132.8	130.0	102		

As noted above, acetaminophen and etoricoxib showed valid test accuracy results.

3.9. Stability of the Drugs in Analytical Solutions

It is critical to understand the level at which analyte solutions are stable. The solution's stability should be tested by storing it at known concentrations at room temperature for 24 h and comparing it with a fresh standard solution. The concentration level of 100% is compared with the reference solution. Table 15 shows the stability findings for fresh samples and 24 h within the specified 98%-102% limit.

Table 15 Stability of acetaminophen and etoricoxib in the diluent solution (The authors)

Drug	**Time**	**Assay %**
Acetaminophen	Initial	100.00
	6 hr	100.25
	12 hr	99.99
	24 hr	99.64
Etoricoxib	Initial	100.00
	6 hr	99.37
	12 hr	98.12
	24 hr	98.01

The given results show that all the assay percentages under all the tested conditions are as per the ICH guidelines. These results indicate that acetaminophen and etoricoxib are stable for at least the specified interval under the test conditions.

3.10. Robustness

This test increases the method's resilience by minor modifications to the process parameters within defined limitations without affecting the findings.

The approach used determines robustness. It is generally accomplished by altering the method parameters and analyzing their impact on the analyte analysis. Robustness was evaluated using solutions developed in the same manner as system or method precision and tested on system suitability characteristics; the results were then compared with those produced using the original procedure. The following individual modifications were implemented:

- Detector wavelength ±5 nm;
- The pH of the mobile phase ±0.1 units from the specified value to assess the impact of pH changes;
- The composition of the mobile phase ±10% organic solvent;
- The temperature +5°C;
- Column change.

Tables 16 and 17 represent the slight variations that were applied to the analytical method to measure the capacity of the method to remain unaffected by minor variations. One analytical concentration was analyzed at each level against the standard solution.

Table 16 Etoricoxib robustness test validation (The authors)

Etoricoxib Robustness				
Sample ID	**Retention time**	**Symmetry**	**Number of theoretical plates (N)**	**Resolution factor**
Robustness +10% organic solvent	6.5	0.997	9657	6.1
Robustness -10% organic solvent	6.5	0.996	9657	6.1
Robustness +5 nm	6.5	0.996	9657	6.1
Robustness -5 nm	6.5	0.997	9657	6.1
Column 1 robustness	6.5	0.995	9657	6.1
Column 2 robustness	6.6	0.998	9657	6.1
Robustness 30C	6.3	0.995	9657	6.1

Continuation of Table 16				
pH +0.1	6.5	0.996	9657	6.1
pH -0.1	6.6	0.997	9657	6.1

Table 17 Acetaminophen robustness test validation (The authors)

Acetaminophen Robustness				
Sample ID	**Retention time**	**Symmetry**	**Number of theoretical plates (N)**	**Resolution factor**
Robustness +10% organic solvent	4.0	0.991	9657	6.1
Robustness -10% organic solvent	4.0	0.991	9657	6.1
Robustness +5 nm (220 nm)	4.0	0.991	9657	6.1
Robustness -5 nm (210 nm)	4.0	0.991	9657	6.1
Column 1 robustness	4.0	0.991	9657	6.1
Column 2 robustness	4.1	0.991	9657	6.1
Robustness 30C	3.8	0.992	9657	6.1
pH +0.1	4.0	0.992	9657	6.1
pH -0.1	4.1	0.991	9657	6.1

The above tables show that the assay's peak parameters are unaffected by the slight variation in the analytical method. This means that the method's capacity remains unaffected by subtle variations. This shows that the method is robust. Fig. 6 represents a chromatogram for the robustness test.

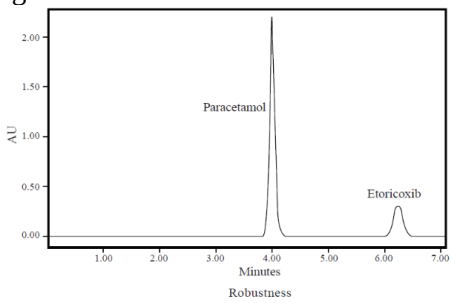

Fig. 6 Chromatogram of acetaminophen and etoricoxib under slight variations of the method in the robustness test (The authors)

The above chromatogram results show no significant change in peak areas, indicating that the analytical method is robust for acetaminophen and etoricoxib.

3.11. Forced Degradation

The drug substance's stress testing helps identify degradation products and the process of breakdown. We subjected the product to high thermal conditions and conducted tests to determine if a breakdown occurred.

Using DSC, we identified the melting temperatures for acetaminophen and etoricoxib separately. Then, we recognized the melting temperature for each ingredient after mixing them, as represented in Fig. 7-9.

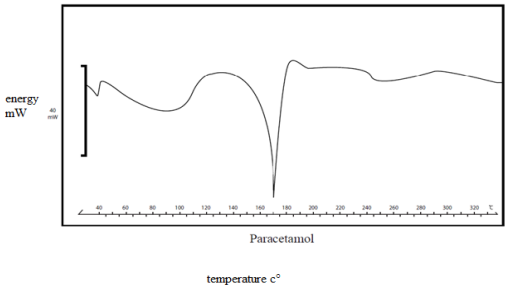

Fig. 7 DSC for acetaminophen (The authors)

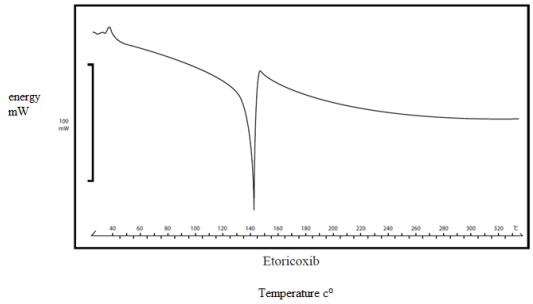

Fig. 8 DSC for etoricoxib (The authors)

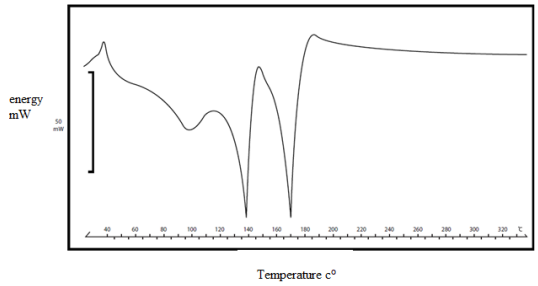

Fig. 9 DSC for acetaminophen and etoricoxib (The authors)

As a result of the DSC analysis in the figures above, we identified the melting temperature for each drug separately; it was 140°C for etoricoxib and 170°C for acetaminophen. The DSC analysis performed on the combination of etoricoxib and acetaminophen shows that the melting temperature for each compound did not change and remains the same compared to the reference of each compound separately. This indicates no interaction or degradation in our combination product.

3.12. Selectivity

The method's selectivity must be investigated to establish the analytical procedure's capacity to measure properly and precisely in the presence of the placebo, active substances, and other components [16]. When injecting a placebo standard, sample, and solvent solution into the column, the parameters of the devised

technique were observed. We found no interference between the analyte, solvent, or placebo, as shown below for each chemical (Fig. 10 and 11).

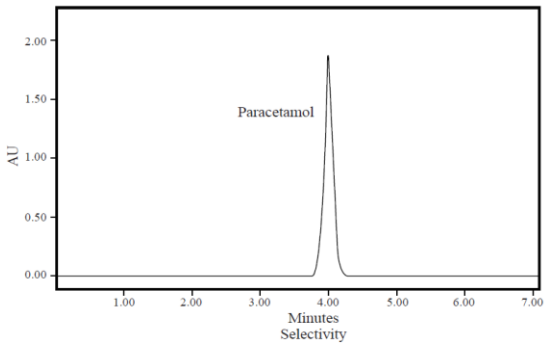

Fig. 10 Selectivity chromatogram for acetaminophen (The authors)

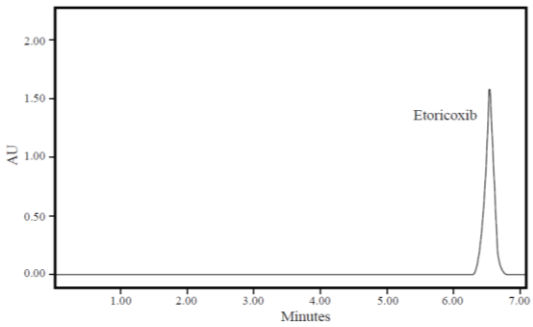

Fig. 11 Selectivity chromatogram for etoricoxib (The authors)

3.13. Placebo Effect

A placebo solution was prepared based on the excipients in a tablet without active ingredients [17]. A placebo solution was prepared by adding the mobile phase and then analyzed in the analytical system. No peaks were observed, suggesting no interaction between the excipients and the active components, as shown in Fig. 12.

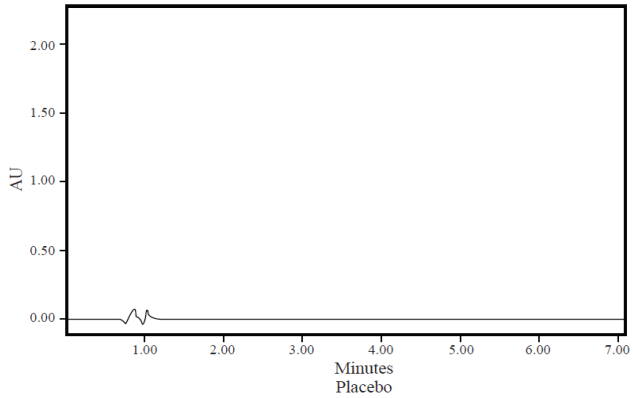

Fig. 12 Chromatogram of placebo formulation (The authors)

3.14. Dissolution Test

The drug dissolution test was conducted to analyze the in vitro drug release data to predict the in vivo drug release profile, and then the samples were validated by HPLC to ensure that they met the requirements in the ICH guidelines. Our tablet was placed in six vessels in the dissolution Apparatus 2. Then, samples were collected after 30 min and analyzed in the analytical system. The observed data (AUCs, RSD %, and accuracy) are listed in Tables 18 and 19.

Table 18 Dissolution test results for etoricoxib (The authors)

Etoricoxib						
Sample ID	**Etoricoxib Area**	**Actual Concentration of Etoricoxib (mg)**	**Theoretical Concentration of Etoricoxib (mg)**	**Accuracy**	**A.v**	**RSD**
Dissolution V1	160524	17.9	18.0	99.2	100.75	1.96
Dissolution V2	167465	18.6	18.0	103.3		
Dissolution V3	162635	18.1	18.0	100.4		
Dissolution V4	158941	17.7	18.0	98.3		
Dissolution V5	162654	18.1	18.0	100.5		
Dissolution V6	166671	18.5	18.0	102.8		

Table 19 Dissolution test results for acetaminophen (The authors)

Acetaminophen						
Sample ID	**Acetaminophen Area**	**Actual Concentration of Acetaminophen (mg)**	**Theoretical Concentration of Acetaminophen (mg)**	**Accuracy**	**A.v**	**RSD**
Dissolution V1	93247	101.1	100.0	101.1	102.47	3.2
Dissolution V2	99544	108.1	100.0	108.1		
Dissolution V3	96641	104.9	100.0	104.9		
Dissolution V4	92145	99.8	100.0	99.8		
Dissolution V5	93254	101.1	100.0	101.1		
Dissolution V6	92211	99.9	100.0	99.9		

According to the ICH guidelines, the accepted limits of accuracy are 98%-102%, RSD % < 6% for the dissolution test.

Results were within the acceptable limits for acetaminophen and etoricoxib, indicating that our compound forms a solution under carefully controlled conditions.

4. Conclusions

The study revealed significant findings on the effectiveness of tablets with etoricoxib and acetaminophen in improving patient compliance. While it aligns with previous research, it also highlights nuances and variations that require further

investigation. The study's implications are multifaceted, offering theoretical insights that can guide future research and practical implications for patients taking tablets with etoricoxib and acetaminophen. It also guides policymakers, practitioners, and stakeholders in making informed decisions. This study underscores the need for ongoing exploration and validation in the field.

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