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Research Article
Combined Paracetamol and Ibuprofen for the Study of Analgesic Activity of Newly Formulated Dose in Prospective Pain Management in Bangladesh

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Abstract

Background and Objective: Pain management has been an area of a great deal of attention for pharmacists for many years. In this study, we report a study which is designed to investigate how a combination of existing effective drugs performs for the relief of pain management. **Materials and Methods:** A combined solid dosage containing paracetamol (500 mg) and ibuprofen (150 mg) is developed and tested under an observational analytical study. The performance of this combined oral solid dosage named maxigesic tablet in order to reduce pain is investigated on the basis of European patent specification. This assay is carried out by employing HPLC system with UV detection at 222 nm. **Results:** The results show the presence of active components to the tune of 112.12% for paracetamol and 101.86% for ibuprofen. The formulated solid dosage is further subjected to separate groups of artificially pain induced mice for a comparative study and it shows more efficacy than single analgesic used in pain management. We observe that the respective potencies for paracetamol and ibuprofenare 98.57 and 102.90%. **Conclusion:** The characterization of both granules and tablets of newly developed formulation demonstrates significant improvement in results of analytical test that not only met the standard specification, but they also reveal that the combined dosage will improve the product quality, efficacy and patient safety in the long run. This trial also shows a significant difference in the percentage of pain inhibition between the two sets of formulations (single and combination of them).

Key words: Paracetamol, ibuprofen, pain management, analgesic activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The relief of pain has been described as a universal human right but is not always easily achieved[1]. Pain is defined by the International Association for the Study of Pain (IASP) as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[2]. In Bangladesh, generally the physicians are keen to prescribe locally available painkillers or analgesics like paracetamol (Acetaminophen), aceclofenac, diclofenac, naproxen, ketorolac, ibuprofen, baclofen etc., for the treatment of mild to moderately severe pain[3]. The use of NSAIDs brings about high risk of having a range of gastrointestinal (GI) problems[4]. Common gastrointestinal ADRs include[5] nausea/vomiting, dyspepsia, gastric ulceration or bleeding, diarrhea etc.[5,6]. However, gastric (but not necessarily intestinal) adverse effects can often be reduced through suppressing acid production by concominant use of a proton pump inhibitor like omeprazole, esomeprazole etc.[7,8]. Use of PPI with NSAIDs may be effective but the pain management cost becomes expensive. Even though the opioid analgesics are effective, they have troublesome and potentially dangerous side-effects which may lead to regulatory and logistical difficulties. On the other hand, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have fewer regulatory restrictions, but they too have important adverse effects that appear only in the cases of higher dose or with longer courses[9]. Prescribing acetaminophen and ibuprofen together is common in clinical practice[10-16]. Paracetamol (Acetaminophen) also known as APAP, chemically named N-acetyl-p-aminophenol is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer)[17,18] with the recommended dose[19] of 4 g day^{-1}. However, in many cases, it does not provide adequate pain relief on its own. On the contrary, ibuprofen [RS-2-(4-isobutyl-phenyl) propionic acid] is one of the most potent orally active antipyretic, analgesic and Non-Steroidal Anti-Inflammatory Drug (NSAID) used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions[20]. Ibuprofen has the advantage of a well-established safety record particularly at doses below 1.5 g day^{-1} in adults[21]. The compound is characterized by a better tolerability and GI safety profile compared with other NSAIDS[21,22]. Besides, paracetamol (Acetaminophen) has traditionally been considered a safe and well-established treatment option in all stages of pregnancy[23] and during breast feeding[24,25]. At least 23 cases are reported in the literature in which infants (ages not stated) were breastfed during maternal use of ibuprofen with no adverse effects reported[26-28]. For the treatment of moderately severe pain, particularly in the cases of dental pain and postoperative pain, NSAIDs are often used in combination with paracetamol (Acetaminophen) resulting in additive and superior pain-relief[10-12,29]. In a cochrane systematic review it was reported that, after surgical removal of lower wisdom teeth the novel combination drug shows encouraging results for pain relief when compared to the single drugs[30]. It had been reported that, a combined dosage form of acetaminophen 500 mg and ibuprofen 150 mg per tablet (Maxigesic, AFT pharmaceuticals, New Zealand) provides superior pain relief after oral surgery compared to the use of acetaminophen or ibuprofen alone and there was no pharmacokinetic interactions between acetaminophen and ibuprofen when administered together[31]. Recently, it is found that, the concomitant administration of ibuprofen and paracetamol in a fixed dose combination (Maxigesic, AFT Pharmaceuticals, New Zealand) does not alter the pharmacokinetic profiles of either drug in the fasted state and there was no effect of food on the absorption from the novel fixed dose combination[32]. However, very limited research has been done of such efficient combination of pain killer and this combination is completely unavailable in Bangladesh. The present study was undertaken to formulate and evaluate the combined efficacy of drugs for the management of pain relief.

MATERIALS AND METHODS

Study design: This study was designed and segmented into two major steps. Firstly, a combined formulation of film-coated (immediate release) tablets containing paracetamol (500 mg) and ibuprofen (150 mg) and consequently the evaluation of quality parameters of the tablet. This was done on the basis of the formulation of paracetamol (500 mg) and ibuprofen (150 mg) of United States Patent Application Publication (Pub. No.: US2011/0275718 A1) and European Patent Specification (Pub. No.: EP1781277 B1) which has been developed by Hartley Campbell Atkinson, founder and owner of AFT Pharmaceuticals Ltd. and evaluation of tablets[33,34]. Secondly, determination of a comparative study of analgesic activity between single dose of paracetamol (500 mg) and the newly formulated combined dose of paracetamol (500 mg) and ibuprofen (150 mg) for the relief of mild to moderately severe pain.

Materials: The API, paracetamol (Pharmatech, Bangladesh) and ibuprofen (IOL Chemicals, India) are collectedto explore the possibility of the formulation of the desired drug. The other excipients such as Avicel-101 (Mingtai Chemicals Taiwan), maize starch (Samyung Genex, Korea), croscarmellose sodium (Mingtai Chemicals, Taiwan), methyl paraben (San Fu Chemicals Ltd., Taiwan), propyl paraben (San Fu Chemicals Ltd., Taiwan), purified talc (Fuji Kajei Co., Ltd., Japan), aerosil 200 (Evonik Industries Ltd., Germany), magnesium stearate (Perter Greven, Netherland) and chemicals such as potassium dihydrogen orthophosphate, sodium hydroxide, HPLC grade acetonitrile, diluted phosphoric acid and dipotassium hydrogen phosphate are obtained from Navana Pharmaceuticals Limited, Dhaka, Bangladesh. All chemicals and reagents are of analytical grade.

Preparation of tablets: According to the study design, APIs and excipients are carefully weighed using electrical balance (Electrolab electrical balance, India) and checked for accuracy. Paracetamol, ibuprofen, Avicel PH-101 and 40% of croscarmellose sodium are mixed together. Starch paste is prepared by suspending maize starch in hot demineralized water and later allowed to cool below 50°C. The paste is added slowly to the powder materials in the mixer to prepare wet mass. Granules are obtained by sieving through a 16 mesh sieve (1.18 mm). Then granules are dried in a hot air oven at temperature 45-60°C until the moisture content becomes 2.4% measured by moisture analyzer (Shimadzu, Japan) and sieved again through 16 mesh sieve. Prior to lubrication and compression, micromeritic properties of the granules are evaluated by angle of repose, bulk density, tapped density, hausner's ratio and compressibility index. The evaluation is done according to the methods specified in USP29-NF24. Blended granules are introduced in tablet compression machine (Pharmachine, India) having 16.5 mm sized die-punch. The compressed tablets are coated using coating material, opadry in coating machine (Hanna Instrument, India). The coated tablets are stored in air tight container at room temperature for further analytical studies.

Evaluation for quality parameters of tablets

Test for friability, hardness and weight variation: The thickness, diameter and length of 10 tablets are determined using slide calipers scale. Weight variation is done by weighing 20 tablets of each batch using electrical balance (Electrolab electrical balance, India) followed by calculation of mean and standard deviation. The friability and hardness of tablet are checked with 10 tablets using friability tester (Roche Friabilator, India) and hardness tester (Monsanto hardness tester, India). All the quality parameters are evaluated according to the methods specified in the United States Pharmacopoeia.

Disintegration test: Disintegration time of tablets is calculated by keeping 6 tablets in each tube of disintegration apparatus (Grovers disintegration tester, India) which is allowed to run at 37±2°C. The time required to break down the tablets into particles and pass through pre-set mesh in tubes is determined.

Assay test: The assay test of paracetamol (500 mg) and ibuprofen (150 mg) within the solid dosage form was determined according to a previously reported work using HPLC system[35]. Standard solution was prepared as, 49 mg of standard paracetamol and 14.5 mg of standard ibuprofen were weighed and taken in a 100 mL volumetric flask and about 60 mL of mobile phase was added to this. The volumetric flask was kept in sonicator for few minutes to dissolve and the volume was filled up to 100 mL with mobile phase (diluent). From this solution 5 mL was taken using pipette in another 50 mL volumetric flask. The volume was filled up to 50 mL with mobile phase. Sample solution was prepared as 10 tablets were weighed accurately (average weight 84.75 mg) and powdered. About 85.2 g of crushed powder was taken in a 100 mL volumetric flask. Sonicator was used to dissolve the powder and the volume was filled up to 100 mL with mobile phase. After filtration, from this solution 5 mL was taken using in another 50 mL volumetric flask and volume was filled up to 50 mL with mobile phase. The sample and standard solution was analyzed at wavelength 222 nm (UV detector) and a flow rate of 0.7 mL min^{-1} for 15 min over RP C18 column (octadecylsilane (ODS), 150×4.6 mm, 5 µm, Phenomenex Inc., Prontosil) in HPLC system (Dionex HPLC system, model-ultimate 3000, invent technology).

Dissolution studies: Dissolution medium (0.2 M potassium buffer pH 7.2) and other dissolution conditions were used according to previously reported study[36]. About 0.2 M potassium buffer (pH 7.2) was prepared according to USP32-NF27. One tablet was dropped into each of the six vessels of dissolution apparatus USP type II (Electrolab dissolution tester, India) containing dissolution medium at 37.1°C and was allowed to run for 60 min. Samples were collected at 5 min interval and analyzed to study dissolution profile at HPLC system at condition of wavelength 222 nm (UV detector) and a flow rate of 0.7 mL min^{-1} for 15 min over RP C18 column (octadecylsilane (ODS), 150×4.6 mm, 5 µm, phenomenex Inc., prontosil) in HPLC system (Dionex HPLC system, model-ultimate 3000, invent technology).

Potency test: Potency test was conducted by introducing the six newly formulated tablets in each of the vessel containing dissolution medium of dissolution tester and it was allowed to run for 1 h. The six samples were withdrawn from each vessel, filtered with filter paper and taken in test tubes. Standard solution was prepared as done in assay test. Then samples were analyzed in HPLC system under the same conditions as assay test against standard solution of paracetamol and ibuprofen[35].

Stability study: In the present study, stability studies were carried out on the combined formulation of paracetamol (500 mg) and ibuprofen (150 mg) wrapped in aluminum foil to prevent the formulation from exposure to light to simulate the aluminum packaging. The study was performed to determine the change in evaluation parameters and *in vitro* release profile on storage carried out at accelerated storage condition at temperature 40°C/75% RH in a humidity chamber for 3 months[37]. Sample were withdrawn after one month interval and evaluated for change in size, shape, color, hardness, friability, *in vitro* drug release pattern and drug content.

Determination of analgesic activity: In order to compare the analgesic activity of the newly formulated drug (paracetamol 500 mg and ibuprofen 150 mg) with the single dose of paracetamol (500 mg) the acetic acid induced writhing test in mice was carried out in healthy male Swiss Albino mice weighing between 30 and 35 g, aged 2 months, were purchased from the Pharmacology Laboratory of Jahangirnagar University, Dhaka, Bangladesh and were individually housed in polypropylene cages in well-ventilated rooms under hygienic conditions with a metal frame lid on its top at standard environmental conditions of temperature 24±1°C. The test protocol was carried out according to the previous study[38].

Animal studies: After one week acclimatization period, the animals are divided into four different groups with ten animals in each. These test animals are marked individually and food is withdrawn 12 h prior to drug administration. The standard solution (paracetamol only) and test solution (paracetamol in combination with ibuprofen) are given orally to the animals of two groups (standard and test groups) by using a feeding needle. After 60 min, pain is induced by intra-peritonial injection of 1% acetic acid (0.01 mL g^{-1} b.wt.) to the animals of three groups (control, standard and test groups) while one group is kept normal without inducing pain. After 5 min elapse time, writhing episodes are recorded for 10 min and the average writhes in each group is calculated. Percentage inhibition of writhing is calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhes (control)-Mean No. of writhes (test or standard)}}{\text{Mean No. of writhes (control)}} \times 100$$

Statistical analysis: For statistical analysis of analgesic activity, GraphPad Prism (version 4.0) computer program (GraphPad Software San Diego, CA, USA) is used for obtaining results and they are expressed as Mean±SEM. A one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* testis used. The statistical method applied in each analysis is described in figures presented in appendix. All the tests are done at the 5% level of significance and for this reason results are considered to be significant when their corresponding p-values are less than 0.05 ($p<0.05$).

RESULTS

Evaluation of granules: The various micromeritic characteristics of the granules are analyzed and showed that the choice of wet granulation method imparted good attributes on granules to formulate good quality tablets. The values of micromeritic parameters with experimental considerations according to USP29-NF24 are shown in Table 1.

The average value of angle of repose and SD, 33.48±0.3863° showed good flow property of granules that resulted in uniform and rapid filling of dies cavities by granulates during tableting. It is ensured by uniformity of tablet's weight shown in Table 2. Hausner's ratio of 1.16±0.006 and Carr's index of 14.08±0.108% are estimated by using values of bulk and tapped densities, respectively.

Table 1: Micromeritics parameters of granules

Micromeritic parameters	Observed outcomes	Experimental consideration
Angle of repose (°, n = 3, ±SD)	33.480±0.3863	31-35 (Good flow property)
Bulk density (g mL^{-1}, n = 3, ±SD)	0.494±0.007	-
Tapped density (g mL^{-1}, n = 3, ±SD)	0.575±0.0078	-
Compressibility (Carr's) index (%, n = 3, ±SD)	14.080±0.108	11-15 (Good compressibility and flow ability)
Hausner ratio (n = 3, ±SD)	1.160±0.006	1.12-1.18 (good flow ability)

Values are Mean±SD of triplicate analyses

Table 2: Evaluation of quality of tablets

Quality parameters	Quality target	Observed qualities
General appearance and organoleptic property	White, smooth tablets without objectionable smell	White, smooth film coated tablets No objectionable smell was found
Size and shape of tablets (mm, n = 10, ±SD)	Tablet size should be patient compliant	Caplet shape tablet with thickness 6.53±0.0048, length 16.55±0.0063, width 8.33±0.007
Weight uniformity (mg, n = 10, ±SD)	Prescribed limit ±5% for tablets weighing more than 300 mg	846.45±1.442
Hardness (kg, n = 10, ±SD)	For film coated tablet 4-10 kg	5.62±0.2821
Disintegration time (sec, n = 6, ±SD)	Not more than 15 min	48±0.89
Friability (%, n = 3, ±SD)	Not more than 1%	0.165±0.0115
Assay test	±15% of label claim	112.19% for paracetamol and 101.86% for ibuprofen
Potency	Within the range of 90.0-110.0%	98.57% for paracetamol and 102.90% for ibuprofen

Values are Mean±SD of triplicate analyses

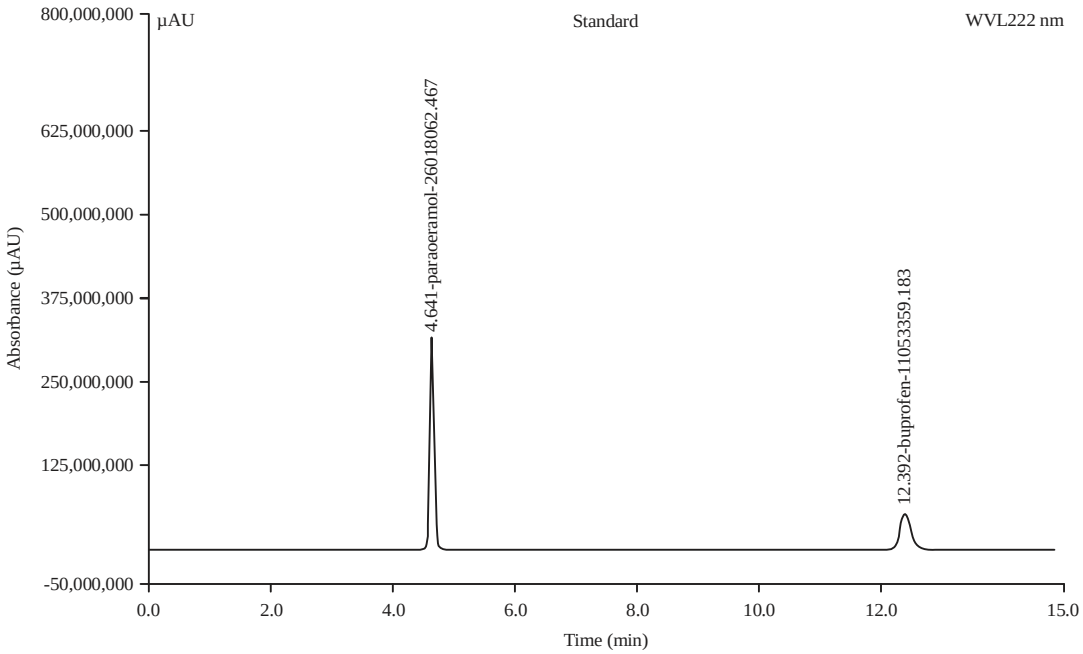
Evaluation of prepared tablets: The quality parameters, specifications of quality target according to USP29-NF24 and observed outcomes of the compressed tablet, paracetamol (500 mg) and ibuprofen (150 mg) are shown in Table 2.

The prepared caplet shape tablets are white, smooth film coated without objectionable odor with average and SD values of thickness: 6.53±0.0048, length: 16.55±0.0063 and width: 8.33±0.007, respectively. Weight variations among all tabletsare obtained and found 846.45±1.442 mg for 10 tablets which is within the range of pharmacopoeial limits and meet the standard specifications. The average hardness of 10 tablets is found 5.62±0.2821 kg as hardness measures the compactness and structural integrity of a tablet under conditions of storage, transportation and handling before usage and according to USP24-NF-24, the hardness for film coated tablet is 4-10 kg which is compatible. The friability is determined and it is 0.165±0.0115%. The friability results are also less than 1%, which meet the standard specifications and show efficiency of formulation development. The average disintegration time of the prepared six tablets is 48±0.89 sec which lie within the specified official limits showing that disintegration time of active ingredients of tablets is not impaired and the tablets disintegrated fast enough for rapid dissolution. The dissolution profile of the combined paracetamol 500 mg and ibuprofen 150 mg tablets is evaluated as the percentage of drug released at 5 min interval for 1 h shown in Table 3. The *in vitro* drug release studies show that approximately 50% of the drug is released from the formulated tablet within 20 min tes and within 1 h, almost 100% of the drug is released. The assay testis conducted and the results show the presence of active components, paracetamol-112.19% and ibuprofen-101.86%. As according to the pharmacopoeial specification, drug content in tablet should be ±15% of label claim, so the new product meet this criterion. This is an important quality parameter to ensure the quality of product and safety of patients and an indication that the formulation process involved in this study is good. The

Table 3: Percentage of drug released at 5 min interval from the tablets

	Percentage of drug released	
Time intervals	Paracetamol (%)	Ibuprofen (%)
Released after 5 min (%)	36.01	25.89
Released after 10 min (%)	43.34	35.00
Released after 15 min (%)	49.00	40.67
Released after 20 min (%)	57.09	48.60
Released after 25 min (%)	67.69	56.89
Released after 30 min (%)	73.45	65.63
Released after 35 min (%)	79.45	69.87
Released after 40 min (%)	87.00	72.24
Released after 45 min (%)	93.67	79.94
Released after 50 min (%)	97.49	85.56
Released after 55 min (%)	100.34	89.23
Released after 60 min (%)	101.02	96.12

chromatogram and data from HPLC of standard paracetamol (500 mg) and ibuprofen (150 mg) obtained for drug content or assay determination are given in Fig. 1 and the chromatogram and data from HPLC of test sample of paracetamol (500 mg) and ibuprofen (150 mg) obtained for drug content or assay determination are given in Fig. 2. From the chromatogram and data obtained by HPLC system, the parameters are analyzed and it is found that the peak of the paracetamol and ibuprofen are sharp. The average retention time with Relative Standard Deviation (RSD) of paracetamol and ibuprofen are 4.600±0.000 and 12.398±0.000, respectively and individual retention times are very close to each other. Areas Under the Curves (AUC) are also close among them and the concentration of the paracetamol and ibuprofen is determined from the values of AUC. The limit for RSD is 2±0.1% and the RSD of assay meets the specification. The potency of paracetamol (500 mg) and ibuprofen (150 mg) in formulated tablets for six samples is shown in Fig. 3. The average potency is 98.57% for paracetamol and 102.91% for ibuprofen. It also meets the specification as the potency should be within the range of 90.0-110.0%. As potency is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity, so the

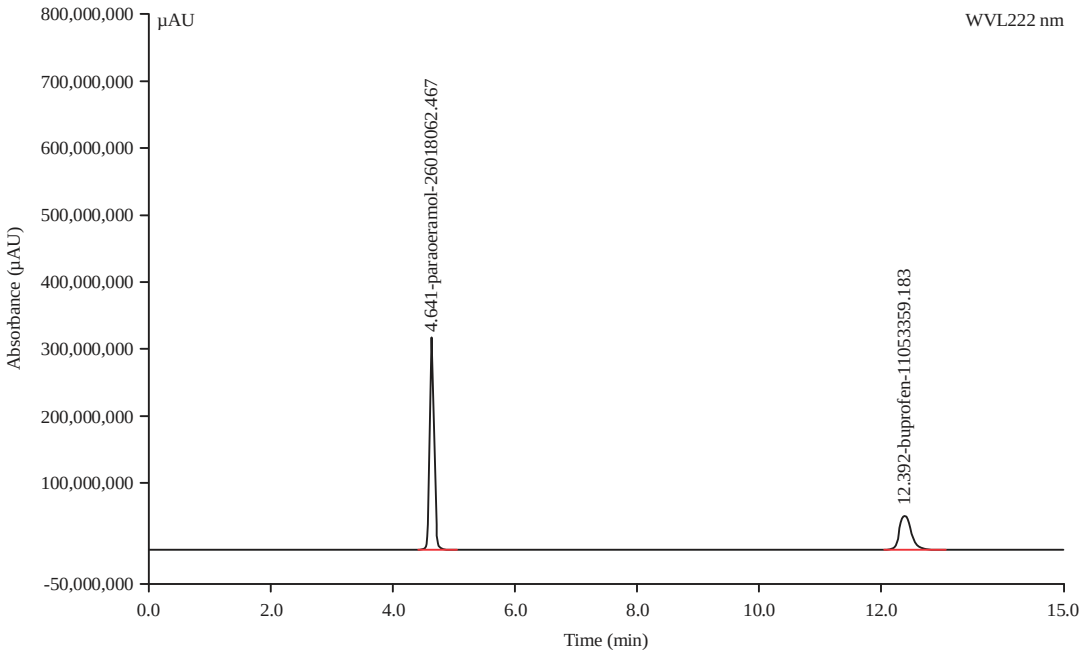


Sample	Sample name	Ret. time	Area	Height	Asynnetry (EP)	Plates (EP)
No		Min	µAU×min	µAU		
		Paracetamol	Paracetamol	Paracetamol	Paracetamol	Paracetamol
		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
4	Standard	4.64	22499539.750	256720050.000	1.11	18406
5	Standard	4.64	22555277.350	285758460.00	1.14	18559
	Average:	4.640	25527408.6	257739255.0	1.1	18482.500
	Rel. Std. Dev:	0.000%	0.175%	0.559%	1.531%	0.585%

Sample	Sample name	Ret. time	Area	Height	Asynnetry (EP)	Plates (EP)
No		Min	µAU×min	µAU		
		Paracetamol	Ibuprofen	Paracetamol	Paracetamol	Paracetamol
		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
4	Standard	12.40	10379208.633	47987930.000	1.25	21571
5	Standard	12.40	10404547.700	47973480.000	1.26	21510
	Average:	12.398	10391878.2	47980705.0	1.3	21540.500
	Rel. Std. Dev:	0.019%	0.172%	0.021%	0.628%	0.200%

Fig. 1: Chromatogram and data from HPLC of standard paracetamol (500 mg) and ibuprofen (150 mg) obtained for assay test

combined tablet will give proper therapeutic effect. The chromatogram and data from HPLC of standard paracetamol (500 mg) and ibuprofen (150 mg) obtained for potency determination are given in Fig. 4 and the chromatogram and HPLC data of test sample of paracetamol (500 mg) and ibuprofen (150 mg) obtained for potency determination are given in Fig. 5. From the chromatogram and data of potency test obtained by HPLC system, the parameters are analyzed and it is found that the peak of paracetamol and ibuprofen are sharp. The average retention time with RSD of paracetamol and ibuprofen are 4.600±0.000 and 11.600±0.000, respectively during potency determination and individual retention times are very close to each other. The AUCs are also close among them and the concentration of the paracetamol and ibuprofen is determined from the values of AUC. In potency determination, the percentage RSD of AUCs is 1.316% for paracetamol and 2.546% for ibuprofen. The limit for RSD is 2±0.1%. Perhaps, the deviation of RSD of ibuprofen occurred due to working error. There are not much differences in results observed in size, shape, color, hardness, friability, *in vitro* drug release pattern, disintegration time, drug content uniformity and before and after the storage period at room temperature and at ambient humidity, but at temperature 40°C/75% RH in a humidity chamber there is a slight change in color of the tablets and small differences in the results of quality parameters. This has been happened due to loss of moisture. This indicates that the formulation is fairly stable at both storage conditions.



Sample	Sample name	Ret. time	Area	Height	Weight	Amount
No		Min	µAU×min	µAU		
		Paracetamol	Paracetamol	Paracetamol		Paracetamol
		UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1
6	Spl	4.64	26018062.467	317646440.000	1.0000	1.1569
7	Spl	4.64	26234221.767	307249980.000	1.0000	1.1665
	Average:	4.643	26126142.1	312448210.0	1.0	1.162
	Rel. Std. Dev:	0.000%	0.585%	2.353%	0.00%	0.585%

Sample	Sample name	Ret. time	Area	Height	Weight	Amount
No		Min	µAU×min	µAU		
		Ibuprofen	Ibuprofen	Paracetamol		Ibuprofen
		UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1
6	Spl	12.39	11053359.183	51055530.000	1.0000	1.0640
7	Spl	12.39	11136062.233	51213780.000	1.0000	1.0720
	Average:	12.393	11094710.7	51134655.0	1.0	1.068
	Rel. Std. Dev:	0.000%	0.527%	0.219%	0.00%	0.527%

Fig. 2: Chromatogram and data from HPLC of test sample of paracetamol (500 mg) and ibuprofen (150 mg) obtained assay test

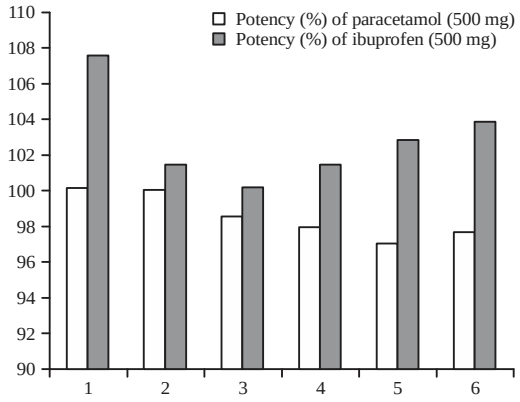
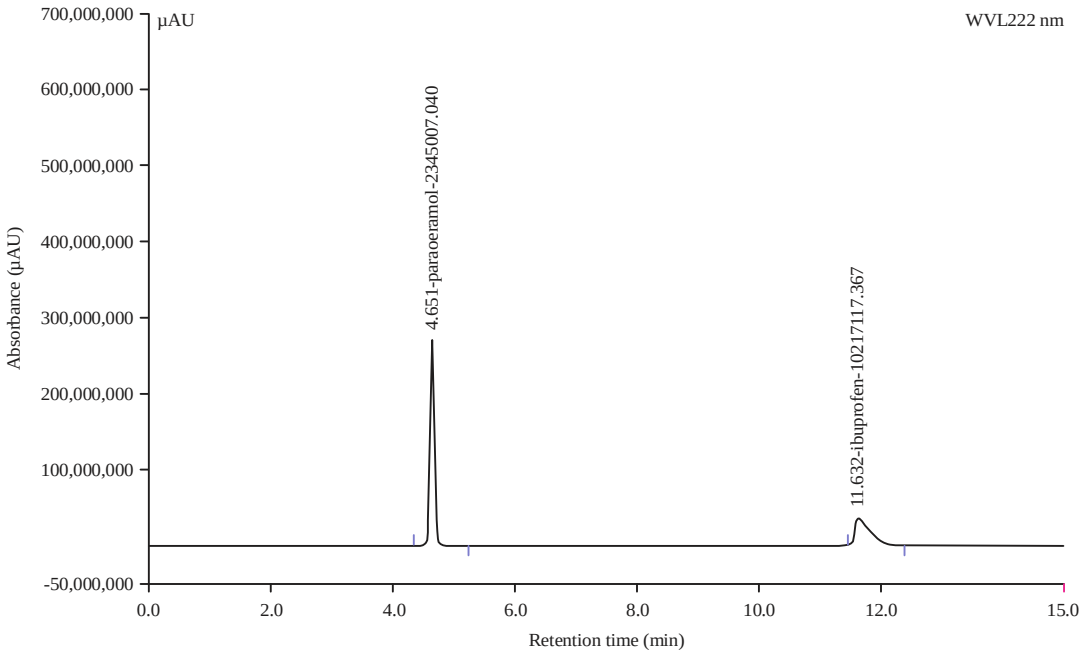


Fig. 3: Graphical representation of potency of paracetamol (500 mg) and ibuprofen (150 mg) in formulated tablets for six samples



Sample	Sample name	Ret. time	Area	Height	Asynnetry (EP)	Plates (EP)
No		Min	µAU×min	µAU		
		Paracetamol	Paracetamol	Paracetamol	Paracetamol	Paracetamol
		UV_VIS_1	UV_VIS_12	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	Standard	4.647	20821735.467	238103005.000	1.168	19006
2	Standard	4.647	20845608.913	239498071.000	1.209	19247
	Average:	4.647	20833672.190	238800538.000	1.189	19127
	Rel. Std. Dev:	0.000%	0.081%	0.413%	2.454%	0.891%

Sample	Sample name	Ret. time	Area	Height	Tailing factor	Theoretical
No		Min	µAU×min	µAU		plates
		Ibuprofen	Ibuprofen	Paracetamol	Ibuprofen	Ibuprofen
		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	Standard	11.633	8684519.840	31421258.000	2.491	11351
2	Standard	11.667	8686660.167	31324984.000	2.490	11387
	Average:	11.650	8685590.003	31373121.000	2.490	11369
	Rel. Std. Dev:	0.202%	0.017%	0.021%	0.040%	0.224%

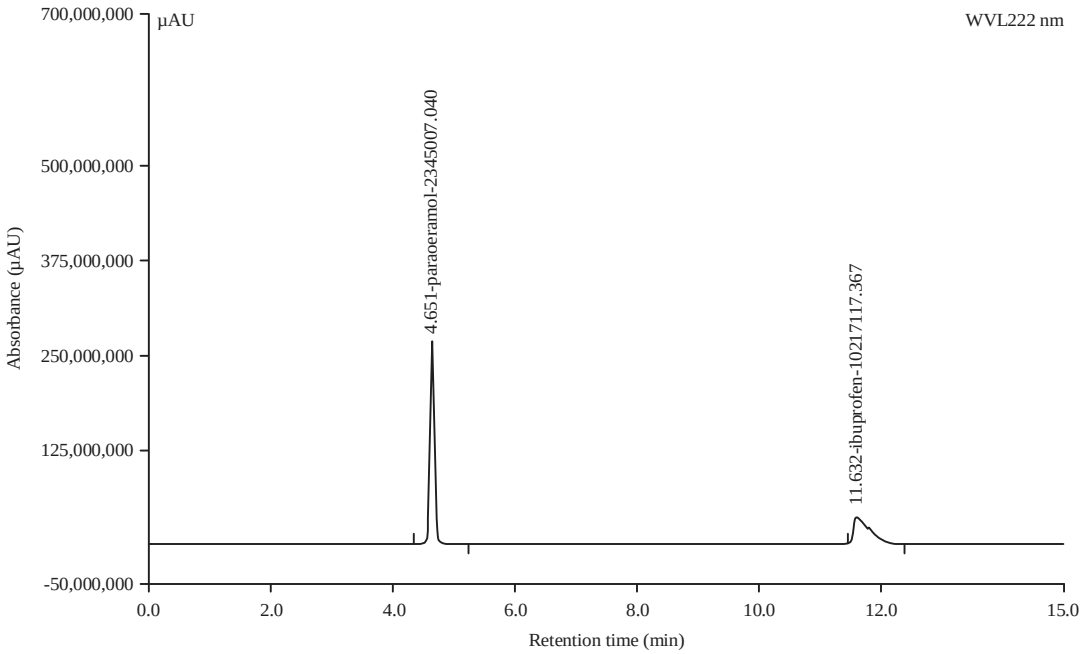
Fig. 4: Chromatogram and data from HPLC of standard paracetamol (500 mg) and ibuprofen (150 mg) obtained for potency determination

Table 4: Analgesic activity by acetic acid induced writhing in Swiss Albino mice

Group	Treatment	Dose (mg kg^{-1})	No. of writhes in 10 min (Mean±SEM)	Inhibition (%)
Control	Water	-	67.3±0.7753	-
Standard	Paracetamol	7.15	33.3±0.7461	50.52
Test	Paracetamol and ibuprofen	9.3	16.7±0.5972	75.19

Data were presented as Mean±SEM, n = 10 in each group, $p<0.05$ compared to pain induced control group and analyzed by using one-way ANOVA followed by Dunnett's test

Determination of analgesic activity of the tablets: Analgesic activity of paracetamol (500 mg) and ibuprofen (150 mg) (Test) in combination and paracetamol (500 mg) alone (Standard) are determined and compared with the only pain induced group (Control) by conducting acetic acid induced writhing test in mice. Analgesic activity by acetic acid induced writhing is given in Table 4. The effects of paracetamol alone and in combination with ibuprofen in acetic acid induced writhing or pain in mice and comparison with the control group are given in Fig. 6. The percentage inhibition of paracetamolis 50.52% and for paracetamol and ibuprofen in combination was 75.19% as graphically shown in Fig. 7.



Sample	Name	Ret time	Area	Height	Weight	Amount
No.		Min	µAU×min	µAU		
		Paracetamol	Paracetamol	Paracetamol		Paracetamol
		UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1
3	Sample	4.600	23450077.040	269568590.000		I.a.
4	Sample	4.600	23417733.403	266994179.000		I.a.
5	Sample	4.600	23066824.523	150769642.000		I.a.
6	Sample	4.600	22925694.487	260498735.000		I.a.
7	Sample	4.600	22861890.013	259119316.000		I.a.
8	Sample	4.600	22707623.550	254715538.000		I.a.
	Average:	4.600	23071640.503	261944333.333	#DIV.0!	#DIV.0!
RSD (%)		0.00%	1.316%	2.072%	#DIV.0!	#DIV.0!
Sample	Name	Ret time	Area	Height	Weight	Amount
No.		Min	µAU×min	µAU		
		Paracetamol	Ibuprofen	Ibuprofen		
		UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1
3	Sample	11.600	10217117.367	35403219.000		I.a.
4	Sample	11.600	9636254.263	33978801.000		I.a.
5	Sample	11.600	9514571.853	33567521.000		I.a.
6	Sample	11.600	9637606.507	33704181.000		I.a.
7	Sample	11.600	9766608.707	34108024.000		I.a.
8	Sample	11.600	9864449.983	34358953.000		I.a.
	Average:	11.600	9772768.113	34186783.167	#DIV.0!	#DIV.0!
RSD (%)		0.00%	2.546%	1.929	#DIV.0!	#DIV.0!

Fig. 5: Chromatogram and data from HPLC of test sample paracetamol (500 mg) and ibuprofen (150 mg) obtained for potency determination

DISCUSSION

Paracetamol demonstrated significant efficacy of analgesia with the comparison to that of ibuprofen but the overall phenomenon was that the combined formulated drug implicated superior analgesic effect for prospective pain management. Although, the analgesic effect of both paracetamol and NSAIDs depends on the type of performing surgery. The present study showed that the combination of paracetamol and ibuprofen (NSAID) exerts strong analgesic

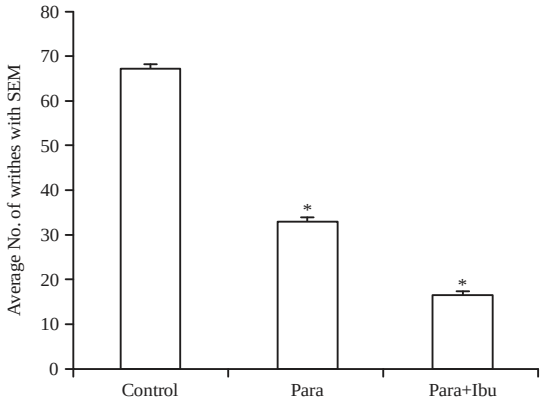


Fig. 6: Effects of paracetamol alone and in combination with ibuprofen in acetic acid induced pain in mice and compared with control. Data were presented as Mean±SEM, n = 10 in each group, *p<0.05 compared to pain induced control group and analyzed by using one-way ANOVA followed by Dunnett's test

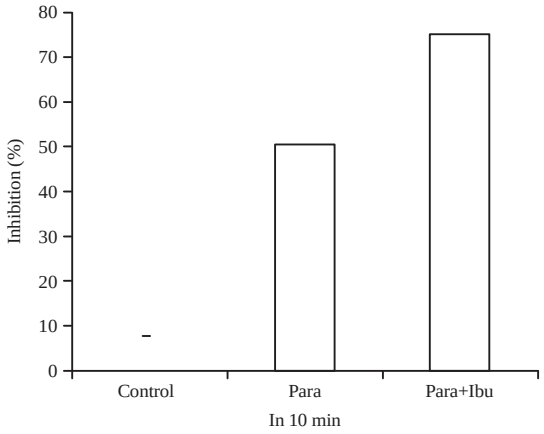


Fig. 7: Graphical presentation of percentage inhibition in acetic acid induced writhing test in mice [Control-no drug, standard-paracetamol (7.15 mg kg^{-1}) and test-paracetamol and ibuprofen (9.3 mg kg^{-1})]

efficacy weather they are used either alone. Paracetamol was known to be found an aniline analgesics class of drug[39]. Previously a study conducted and found that best tablet properties were obtained with coated paracetamol and exhibits improved flowability and adequate compressibility. Furthermore coated paracetamol in combination with both investigated superdisintegrants shows faster disintegration times and dissolution rates in comparison to paracetamol of direct compression. With regards to results, coating of paracetamol particles has a positive effect on manufacturing of tablets with immediate release[40]. Paracetamol is prescribed to relieve pain alone the reduction of fever whose chemical structure is known as an N-acetyl-p-aminophenol. The Non-Steroidal Anti-Inflammatory Drug (NSAIDs) ibuprofen is known as a (±)-2-(p- isobutyl phenyl) propionic acid which is prescribed to relieve fever, pain and inflammation[41]. Perioli *et al.*[42] conducted a study to improve FURO biopharmaceutical properties by its formulation in a new solid oral dosage form. The result indicated that the solid oral dosage form proposed represents an interesting tool to improve FURO biopharmaceutical properties because of the *in vitro* high and almost complete drug release observed. This means that the combination of the composite MgAl-HTlc-FURO with a suitable super disintegrant produces a synergic effect responsible for FURO release improvement. The combined (paracetamol+ibuprofen) formulated drug demonstrated significant ($p<0.05$) analgesia which reduced the number of writhes in mice induced by intra-peritonial injection of 1% acetic acid (0.01 mL g^{-1} b.wt.). The combined formulated drug seems to be found the significant analgesic effect as compared with paracetamol standard. Many expert researchers recommend that the use of combined analgesics[43] in the pain management which is consistent to our conclusion. Whatever the previous study[44] supported the expectation that such combined formulated drug exerts considerably less adverse effect compared to either of its component. The findings of this research study are so important, because different types of clinical studies are consistent in which the combined formulation of paracetamol with ketoprofen or diclofenac, different surgical procedures were associated with the lower pain scores than paracetamol alone[10]. The available clinical data of analgesia obtained from the comparison between the paracetamol/NSAIDs combination and NSAIDs alone suggested that the standard doses of paracetamol increase the efficacy of analgesia with the addition of ketoprofen, diclofenac or naproxen[45]. This result supports the effect of analgesia of the formulated combined drug when treated with the experimental animals. Another study was perfomed for the formulation and evaluation of quetiapine immediate release film coated. The results indicated that there were insignificant changes during studies[46]. Hence, the results suggest the feasibility of developing immediate release tablets consisting of quetiapine, which has an excellent tolerability profile offering high patient acceptability that may promote patient adherence to medication and an improved quality of life. Peripheral COX enzymes are the most important enzymes involved in the pain management which are inhibited by the treatment with the combined formulated drug. Interestingly, paracetamol is an important inhibitor of peripheral COX enzymes and inhibits COX enzymes strongly to relief pain[47].

Several studies suggested that the paracetamol is a potent inhibitor of the peripheral COX enzyme in which one of these studies reported about the preferential COX-2 inhibition in volunteers receiving 1000 mg paracetamol[48]. Interestingly, it can be noted that the central action site of paracetamol was considered as COX-3 and a splice variant of COX-1 but the selective interaction is found unlikely to be clinically relevant[49] and the mechanism of paracetamol-induced analgesia implicates the affection in some different way on COX-1 and/or COX-2[50]. Badawy *et al.*[51] conducted a study which purpose was to investigate the specific mechanism by which elevated gastric pH reduces the absorption of BMS-561389, a factor Xa inhibitor and to develop a solid formulation strategy to overcome this gastric pH interaction[49]. The result indicated a multitier approach was successful in identifying a solid dosage form that minimizes the pH-dependent absorption of this drug candidate[51]. On the other hand, several research studies supported that the various types of NSAIDs addition enhance the pain relieving effect comparable to the acetaminophen alone. It is published that acetaminophen in combination with NSAIDs (ibuprofen, acetylsalicylic acid [ASA], naproxen sodium and diclofenac potassium) is very effective for the relief of mild to moderately severe migraine pain[52]. Different doses of the combination of paracetamol and ibuprofen is found to provide safe superior pain relief to placebo in adult patients following third molar removal surgery in clinical practice[53]. Moreover, the most important evidence is that our data adds strong analgesic effect of the formulated combined drug supported that this combination is superior to ibuprofen or paracetamol alone. During study, different types of manufacturing problems were found and solved after studying and analyzing different formulations. In formulation aspects, it is important to note that the physical and chemical characteristics of newly formulated tablet can be changed from lower to higher level if different excipients are used.

CONCLUSION

The present study is aimed to formulate a combined solid dosage of paracetamol (500 mg) and ibuprofen (150 mg). The newly formulated tablets meet the compendial limit in terms of various pre-formulation and post-formulation quality parameters. The data of the present analgesic study demonstrates that paracetamolin combination with ibuprofen produces a synergistic analgesic effect. It may be noted that the doses of paracetamol and ibuprofen are small and if they are compared with those referred in the literature, it is possible to suggest that the combination of paracetamol (500 mg) and ibuprofen (150 mg) will be effective for the clinical treatment of mild to moderately severe pain such as acute migraine pain, dental pain, post-operative pain etc in prospect of Bangladesh. In addition, it is demonstrated that the effect of the combined paracetamol and ibuprofens is superior to that of either component alone. Therefore, these mixtures are viable alternativesfor clinical pain management, especially because the low doses of the components with greater efficacy may be a potential index of lower incidence of adverse effects and patient safety in long run. Further study may include modification in the development of formulation along with clinical trials in future.

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