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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR ESTIMATION OF MEPHENESIN AND IBUPROFEN

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Keywords:

Mephenesin, Ibuprofen, HPLC, Validation

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ABSTRACT: A new RP-HPLC method was developed for Mephenesin (MEP) and Ibuprofen (IBU) and validated as per ICH guidelines. Good chromatographic separation of Mephenesin and Ibuprofen was achieved by using Agilent C_{18} column (150mm×4.6mm, 3.2µ p.s). The system was operated at ambient temperature using a mobile phase consisting of Acetonitrile, 0.01M potassium dihydrogen phosphate pH 3.0 (60:40 v/v) isocratically at a flow rate of 0.5 ml/min. Detection was carried out at 212 nm and retention time was 3.4 min and 9.3mins respectively. Linearity was achieved from 10-40 µg/ml for Mephenesin and 2-8µg/ml for Ibuprofen with r^2>0.99. The analytical method validation studies were performed as per International Conference on Harmonization-Quality (ICH-Q2 (R1)) guidelines. The method was efficiently validated with acceptable accuracy, specificity and precision for the estimation of Mephenesin and ibuprofen. It also aimed to apply the developed and validated method, for the analysis of drug release studies in marketed formulation (gel X) containing Mephenesin and Ibuprofen.

INTRODUCTION: Mephenesin (MEP) (**Fig.1**), 3-(2-methylphenoxy) 1, 2-propanediol, is a centrally acting skeletal muscle relaxant and is used in the treatment of moderate to severe muscle spasm. Its relaxant action is shown by blocking the internunical neuron of the spine which then modulates reflexes maintaining the muscle tone [1]. Ibuprofen (IBU) (**Fig.2**), (*RS*)-2-(4-(2-Methylpropyl) phenyl) propionic acid is a non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid used for relieving pain, fever and inflammation.[5, 10, 12,13]

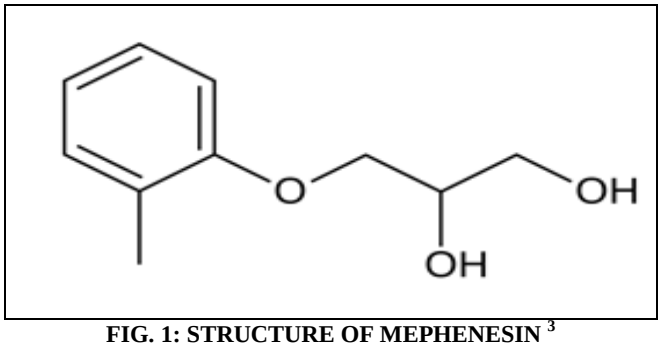

FIG. 1: STRUCTURE OF MEPHENESIN [3]

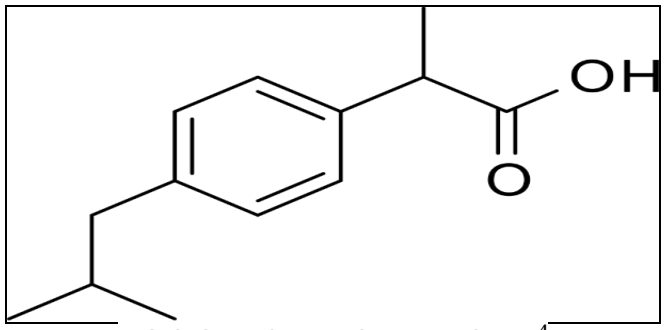

FIG. 2: STRUCTURE OF IBUPROFEN [4]

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Inflammation is one of the condition which is often associated with acute muscle spasm. Hence, a combination of IBU, a non-steroidal anti-inflammatory drug along with MEP, a skeletal muscle relaxant. From the literature survey, many methods were found to be reported for estimation of MEP and IBU individually and in combination with other drugs.

A UV method developed for estimation of MEP and IBU in combination. Linearity, precision and accuracy of the proposed method was estimated by analyzing commercial as well as gel samples[5].Another method is reported in which stability indicating HPLC method was developed for simultaneous determination of MEP and Diclofenac diethylamine. This method was found to be simple, specific, accurate and stability-indicating for determination of MEP and Diclofenac diethylamine which can be successfully employed for simultaneous quantitative analysis of MEP and Diclofenac diethylamine in bulk drugs and formulations [6]. An article demonstrated formulation and evaluation of MEP topical gel using different polymers as gelling agents, permeation enhancers like propylene glycol and dimethyl sulfoxide(5-15%) in various concentrations (5-15%). The prepared gels were evaluated for drug content, physical appearance, pH, extrudability, spreadability, skin irritation to observe toxicity or side effects and also for anti-inflammatory activity [7].

However, there was no method found where MEP and IBU were analyzed in combination by HPLC. Hence efforts are directed to arrive at a simple, sensitive, accurate and reproducible method for simultaneous estimation of these two drugs and its dosage form.

2. MATERIALS AND METHODS:

2.1 Drug and chemicals:

MEP and IBU were obtained as a generous gift samples from Samanta Organics Pvt Ltd. Boisar and Flamingo Pharmaceuticals respectively. HPLC grade Acetonitrile (ACN), Methanol, orthophosphoric acid, triethyl amine and Acetone were obtained from S.D fine chemicals. Ultipore 0.45µm Nylon 6 filter was obtained from Pall Corporations.

2.2 Instrumentation:

HPLC system consisted of Agilent 1260 series, a photodiode array detector (DAD) equipped with Agilent pump. Open lab control panel (ezchrome) software was used for data integration and 20µL loop was used for injections.

2.3 Site of Experiment:

Experiment was performed in the labs at SVKM's Dr. Bhanuben "Nanavati College of Pharmacy, Vile Parle West, Mumbai in the year 2015 in June.

3. EXPERIMENTAL:

3.1 Chromatographic conditions:

The HPLC seperation was performed using Agilent 5-RP C_{18} column (150mm × 4.6mm, 3.2µm). The isocratic mobile phase consisting of Acetonitrile (ACN): 0.05M Phosphoric acid buffer pH 3.0 (adjusted with OPA) (60:40 v/v) was delivered at the flow rate of 0.5mL/min. Prior to use the mobile phase was filtered through Ultipore 0.45µm Nylon 6 filter and degassed by sonication in an ultrasonic bath. Detection wavelength was set to 212nm and the column temperature was maintained at room temperature.

3.2 Preparation of buffer:

Different pH buffers were used during the experiment. Phosphate buffer 0.05M was prepared by weighing 6.8gm of potassium dihydrogen phosphate in 1000ml volumetric flask, making up the volume by Millipore water and maintaining the pH 3.0 with orthophosphoric acid.

3.3 Preparation of standard solution:

The standard solutions were prepared by weighing 10mg of MEP and IBU respectively in different volumetric flasks and dissolving in methanol and obtaining the final concentration of 100µg/ml and further diluting to appropriate concentration. After filtration through Ultipore 0.45µm Nylon 6 filter, aliquots were combined to get a mixture of MEP and IBU. This mixture was further diluted with mobile phase before injection into HPLC system.

3.4 Method Validation:

The developed analytical method was validated for linearity, accuracy, precision, specificity, LOD and LOQ. All validation experiments were designed according to the principles out lined in the ICH Q2guidelines.[3, 14]

3.4.1 System suitability:
System suitability parameters were evaluated to verify that the analytical system is functioning properly and can give accurate and precise results. Peak asymmetry factor, tailing factor, resolution between MEP and IBU were the parameters evaluated.

3.4.2 Linearity:
Linearity studies were performed according to the ICH guidelines. It was established by plotting calibration curves (n=3) using standard solutions of MEP and IBU and diluting to concentration range of 20–80 µg/ml and 2-8 µg/ml in methanol and respectively with mobile phase. At least six concentrations of the solution were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak area versus its corresponding. Values of coefficient of regression, slope and Y-intercept of the calibration curve were calculated.

3.4.3 Precision:
Precision was studied by conducting repeatability, intermediate (intra-day) and reproducibility studies. A known concentration of MEP and IBU were tested. For intra-day precision, the mixed standards solution was analyzed for six times within a day, while for reproducibility studies, it was examined in duplicates for three consecutive days. Chromatography was performed and the variations were expressed in % RSD.

Repeatability was confirmed by analyzing the mixture as above mentioned procedure by HPLC with six determinations in triplicate. The %RSD was calculated of the obtained data.

3.4.4Accuracy:
Accuracy of the method was evaluated using recovery studies. A known amount of API was added to standard solutions and analyzed using the method described above. Test was performed in three replicates at the concentration level of 80, 100 and 120% to evaluate the accuracy of the proposed method.

3.4.5Specificity:
Specificity is the ability of the method to measure the analyte in the presence of other relevant Components those are expected to be present in a sample. In specificity studies, separation and resolution was observed between standard solution of MEP and IBU and its placebo solution.

3.4.6 LOD and LOQ:
LOD and LOQ were determined using the signal-to-noise ratio by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3:1 is generally considered acceptable for estimating the detection limit while for quantification limit a ratio of 10:1 is generally considered acceptable

3.4.7 Robustness:
Robustness studies of the analytical procedure were performed to ensure the validity is maintained whenever used. The method was performed with little variations like changing mobile phase and changing the flow rate of the mobile phase (±0.2 ml/min). Chromatograms of six replicas of the mixture solution were obtained and effect of each deliberate change was evaluated by applying system suitability parameters for each deliberate change and calculating value of %RSD.

3.5 Application of the developed method:
The optimized chromatographic method was applied on the marketed formulation (gel X) and assay of the formulation was performed. 0.5gm of the gel was weighed and dissolved in 10ml of methanol and filtered through Ultipore 0.45µm Nylon 6 filter and the resulting solution was diluted further with mobile phase and injected into HPLC.

3.6 Diffusion studies:
The diffusion studies of marketed gel were conducted and evaluated by obtaining chromatograms by HPLC. The studies were performed using Frank diffusion cell apparatus containing 6 cells with receiver compartment capacity of 22ml each. A membrane of 0.45 µm was used for diffusion. The medium used for diffusion of the gel was finalized after different trials and buffer 6.8 pH (having a pH 6.8 of the skin membrane) was optimized. The medium, before processing, was degassed via sonication process, and temperature was set at 37 °C during the experiment. At appropriate time, 1 mL of the sample was withdrawn from the receiver

compartment and the same amount of fresh solution was added to keep the volume constant. Release was recorded for 0–240mins.

4. RESULTS AND DISCUSSIONS:

4.1 Separation and identification: To develop as simple and rapid HPLC method for the determination of active components of MEP and IBU is the aim of the study. During the method development, the complete separation of the two analytes of interest, and good peak shapes was the top priority. Different mobile phases like, methanol–water, methanol–phosphoric acid and acetonitrile–water, were choseninan isocratic elution, these separations or the peaks hapes of the two analytes of interest were not satisfactory. After trial and error, an isocratic elution of Acetonitrile-phosphoric acid buffer pH 3.0 (60:40, v/v) was finally used to achieve complete separation of the two analytes. Selecting 212nm as the detection wavelength resulted in acceptable response and enabled detection of the two compounds. Elution was carried out at the flow rate of 0.5ml/min. Separation parameters are summarized in **Table 1** and **Fig. 3** show the chromatogram of MEP and IBU in a mixture respectively.

TABLE 1: SYSTEM SUITABILITY RESULTS OF THE PROPOSED HPLC METHOD FOR SEPARATION OF MEP AND IBU IN MIXTURE

Solution	Composition	System suitability parameters			
		Capacity factor(K')	Resolution (Rs)	Theoretical plates	Tailing factor
MEP and IBU standard mixture	MEP	3.407	-	5589	0.88
	IBU	9.327	20.72	9196	1.48
Acceptance criteria		0.5 > X < 15	> 2	> 2000	< 2.0

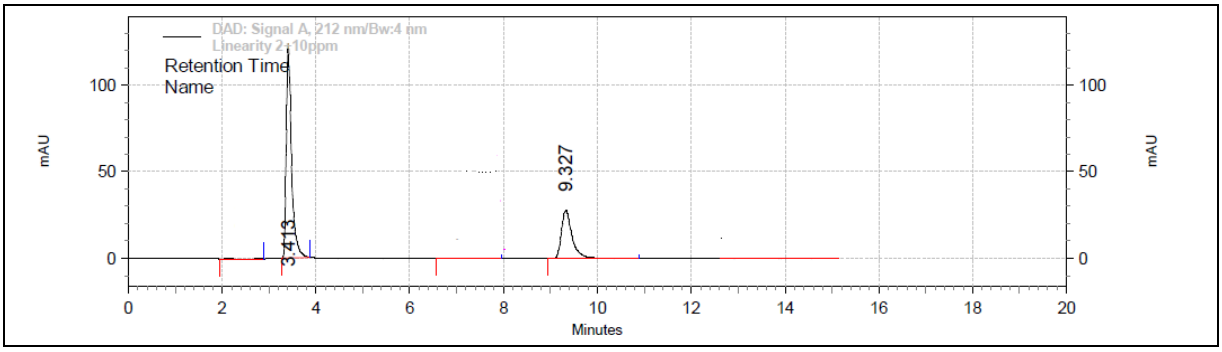

FIG. 3: HPLC CHROMATOGRAM OF THE MIXTURE OF MEP AND IBU

4.2 Validation of the developed method:

4.2.1 System suitability test: The capacity factors (K') of MEP and IBU were 3>K'<9 and the resolution between their peaks was higher than 2.5. The plate count was more than 5000 and their symmetry factors were in between 0.88 and 1.48. The results are summarized in **Table 1**.

4.2.2 Specificity: Specificity can be described as the capability of the method to accurately measure the response of the two analyzed compounds without any interference. HPLC chromatograms were recorded separately for MEP and with IBU, blank and placebo preparations which displayed a single, non-overlapped peak as shown in **Fig. 3, 4, 5.**

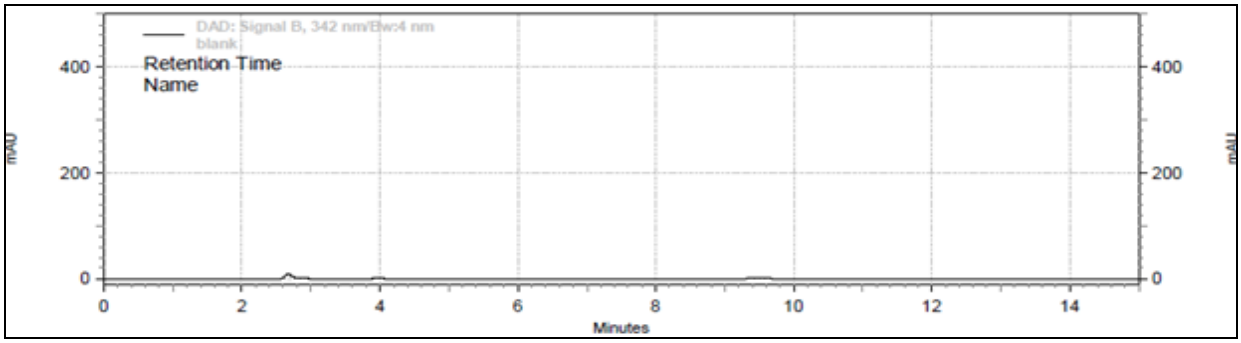

FIG. 4: HPLC CHROMATOGRAM FOR PLACEBO RUN

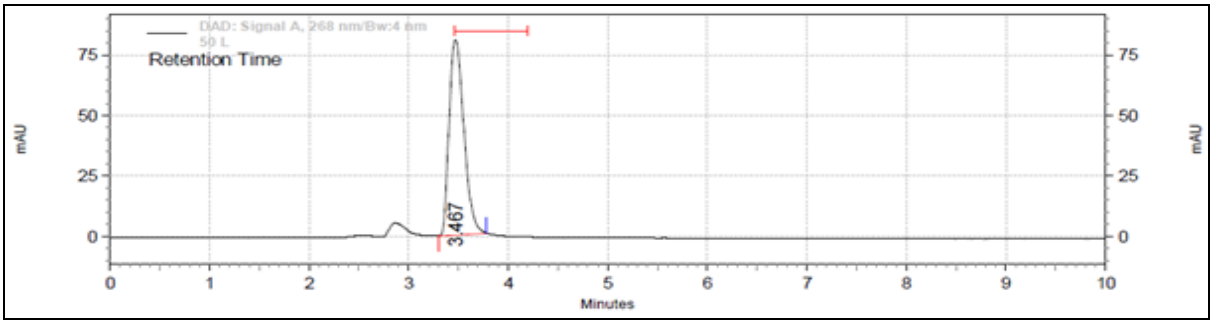

FIG.5: HPLC CHROMATOGRAM FOR MEP

4.2.3 Linearity: A series of calibration solutions were prepared. The calibration curves were found to be linear for MEP in the range of 10-40µg/ml and 2-10µg/ml for IBU. Samples were injected in triplicate. Correlation coefficient were found more than 0.99. **Table 2** enlists the linearity parameters of the calibration curve for MEP and IBU in sample solution.

TABLE 2: LINEARITY PARAMETERS FOR MEP AND IBU

Drug	Range (µg/mL)	Regression equation	Correlation coefficient (R^2)	LOD (µg/mL)	LOQ (µg/mL)
MEP	10-40	y = 179786x + 185433	0.998	1.59	5.30
IBU	2-8	y = 171877x + 558989	0.997	0.37	1.26

4.2.4 Precision: The precision parameters of repeatability, intermediate precision and reproducibility are shown in **Table 3**. The percent relative standard deviation (%RSD) was calculated, which was found to be less than 2% for both the drugs, indicating that the method was reliable and reproducible.

TABLE 3: PRECISION PARAMETERS FOR MEP AND IBU

Drugs	Precision parameters		
	Repeatability (%RSD)	Intraday precision(%RSD)	Reproducibility(%RSD)
MEP	0.36	1.51	0.21
IBU	1.29	1.60	1.90

4.2.5 Accuracy: The results are expressed as percent mean recoveries for MEP and IBU in the samples. From the results depicted in **Table 4**, the method recoveries in range for MEP and IBU at 80, 100 and 120% and thus was found to be accurate.

TABLE 4: ACCURACY STUDIES FOR MEP AND IBU.

Accuracy level (%)	MEP		IBU	
	Mean Recovery (%)	%RSD	Mean Recovery (%)	%RSD
80	102.01	1.53	96.34	1.58
100	98.46	1.00	99.11	1.56
120	95.44	1.26	99.42	1.16

4.2.6 LOD and LOQ: LOD is the concentration of analyte that produces analytical signal equal to thrice the deviation of back ground signals. The LOQ is the lowest amount of analyte which could be quantified. These parameters were studied for MEP and IBU. The LOD and LOQ for MEP and IBU are tabulated in **Table 1**.

4.2.7 Robustness: Robustness of the method was checked after deliberate alterations of mobile phase composition and flow rate shows that the changes of the operational parameters do not cause significant change in the performance of the chromatographic system; results are displayed in **Table 5**. Tailing factor for MEP and IBU was found to be below 2 and the components were well separated. The %RSD of MEP and IBU were below 2 and did not show a significant change when the critical parameters were modified. Considering the results after modifying the system suitability parameters and the specificity of the method, it was to be concluded that the method conditions are robust.

TABLE 5: ROBUSTNESS PARAMETERS FOR MEP AND IBU

Level	Flow rate (ml)	MEP		IBU	
		Retention time (mins)	%RSD	Retention time (mins)	%RSD
-1	0.4	4.29	1.76	11.95	0.39
0	0.5	3.53	0.12	9.32	0.05
+1	0.6	2.82	1.18	7.88	0.58

4.2.8 Assay of marketed formulation:

The proposed validated HPLC methods were applied to the determination of MEP and IBU in (Acks gel) pharmaceutical gel. **Table 6** shows the mean percentage drugs found and the RSD% values indicating that the proposed validated HPLC methods could be adopted for the selective determination of the investigated drugs in their pharmaceutical preparations without interference from their corresponding degradation products. Representative chromatograms are illustrated in **Fig. 6**.

TABLE 6: ASSAY PARAMETERS FOR PHARMACEUTICAL PREPARATION

Pharmaceutical preparation	MEP		IBU	
	% Mean recovery	%RSD	%Mean recovery	%RSD
Gel X	100.45	0.088	101.38	1.37

4.2.9 Diffusion studies: At time intervals of 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 and 240 min (n=3, samples were drawn at each time interval), the release rate of marketed gel dosage form having MEP and IBU was noted. **Fig.7**. shows the release rates of the drugs from the marketed formulation. Retention time was found to be 3.24 and 9.58 min for MEP and IBU respectively (**Fig. 6**) that matched with the retention time of the optimized chromatogram. Thus the developed method can also be used for routine analysis of the combination in marketed gel. Sample chromatogram obtained at 10 min time interval is shown in **Fig. 6**.

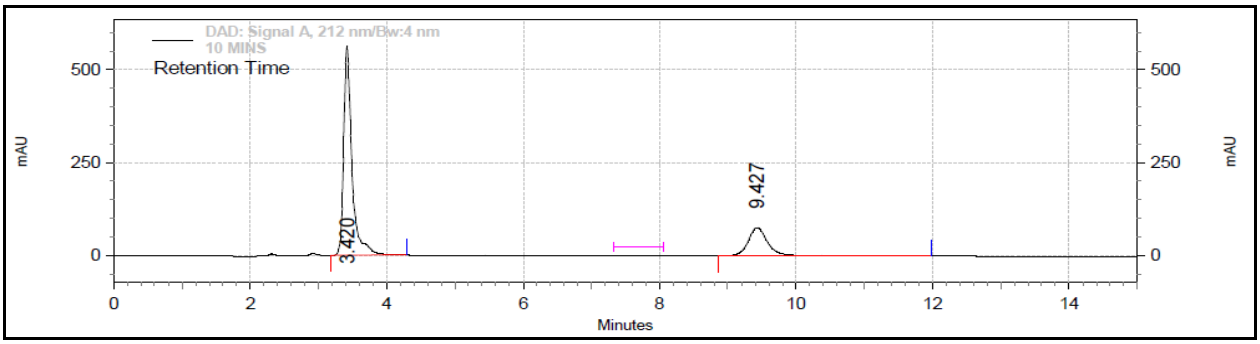

FIG. 6: CHROMATOGRAPH FOR DIFFUSION STUDIES OF THE MARKETED FORMULATION

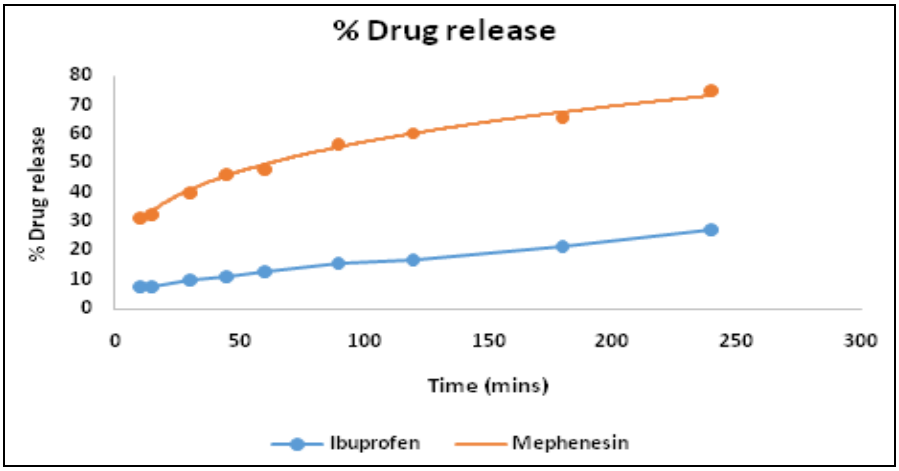

FIG. 7: DRUG RELEASE PROFILE FOR MEP AND IBU

CONCLUSION: The optimization of RP–HPLC method showed that the mobile phase composition, pH and flow rate were more crucial parameters to be controlled for reproducible and quantitative estimation of MEP and IBU. The developed RP–HPLC method could further be applicable for method development method or in combination. The validated developed RP–HPLC were found simple, specific, accurate, rapid, precise, economical and reliable for contemporary analysis of drugs in gel formulation. Diffusion procedure was performed to characterize the drug release rate for gel formulation and further analyzed by developed RP–HPLC method. The diffusion and developed method for estimation could be applied to routine quality control analysis of MEP and IBU.

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Competing Interests: The authors declare that they have no competing interest.

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