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A VALIDATED RAPID HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF FAMOTIDINE AND IBUPROFEN FROM PHARMACEUTICAL FORMULATION

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ABSTRACT

A high performance thin-layer chromatography method was developed and validated for simultaneous estimation of Famotidine and Ibuprofen in combined dosage form. The aluminum backed plates coated with 0.2-mm layers of silica gel 60 F254 having size of 80 mm × 100 mm was used for separation. The mobile phase was Chloroform: Methanol: Toluene: Ammonia in the ratio of 3:1.2: 1: 0.1% v/v/v/v and detection was done at 280 nm in Densitometric detection. The FAM and IBU showed linearity in the range of 15-40 ng/spot and 300-1200 ng/spot respectively. the method was found to be precise and % RSD was less than 2.0 for intra-day, inter-day and intermediate precision study. The method was able to recover FAM and IBU in the range of 98.72-101.66% and 98.45-101.65% respectively with % RSD less than 2.0. The method was successfully used to analyzed FAM and IBU in tablet dosage form.

Keywords: famotidine, ibuprofen, HPTLC, simultaneous estimation, validation, combined dosage form.

Short Title: HPTLC method for simultaneous estimation of FAM and IBU

INTRODUCTION

Ibuprofen (IBU), a non-steroidal anti-inflammatory drug (NSAID) and chemically (RS)-2-(4-isobutylphenyl)propionic acid, has been used in humans for nearly forty years. While generally regarded as safe, ibuprofen and other NSAIDs can cause gastric irritation, which may range from simple discomfort to ulcer formation. This effect is generally thought to be related to the inhibition of fatty acid Cyclooxygenase (COX) enzyme, and thus inhibition of the production of prostaglandins.[1] This side-effect is a particular problem for individuals who take ibuprofen for extended periods of time, such as patients suffering from rheumatoid arthritis and osteoarthritis.[2]

Famotidine (FAM) is a Histamine type 2 (H2) receptor antagonist and chemically it is 3-[[2-[(Amino iminomethyl) amino]-4-thiazolyl]methyl]thio]-N-(aminosulfonyl) propanemidami de. The histamine H2 receptor antagonists competitively inhibit histamine actions at all H2 receptors, but their main clinical use is as inhibitors of gastric acid secretion. These agents not only decrease both basal and food-stimulated acid secretion by 90% or more, but numerous clinical trial indicate that they also promote healing of duodenal ulcers.[1] Gastric emptying time, pancreatic or biliary secretion, and lower esophageal pressures are not altered by Famotidine.[3]

The combination of FAM and IBU can be used for the treatment for osteoarthritis and rheumatoid arthritis patients who are at risk for upper gastrointestinal ulcers [4]. During clinical trial, combination demonstrated a statistically significant reduction in the incidence of upper gastrointestinal ulcers versus treatment with ibuprofen alone [4]. The structures of the two drugs are shown in Figure 1.

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Monographs of IBU and FAM, as pure drug substances and its formulations are available in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). Pharmacopoeial monographs of IBU or FAM mention titrimetric [5] or HPLC [5, 6] methods for assay in bulk or in formulations.

A survey of literature revealed that titrimetric,[5] spectrophotometric,[7, 8] spectrofluorimetric[9], FTIR[10] and HPLC[11-13], HPTLC[14-16] methods have been reported for estimation of IBU in bulk, formulations and combination with other drugs. The titrimetric[5], spectrophotometric [17-20], spectrofluorimetric[21], HPLC[5-6, 22-26] and HPTLC[27-29] methods have been reported for estimation of FAM in bulk, formulations and combination with other drugs. Development and validation of liquid chromatographic method for estimation of IBU and FAM in combined dosage form have been reported by Shah et al. [30] However, this reported method requires long run time while present method gives good separation with better peak shape in comparatively shorter run time of 5 min.

The present study involves development and validation of high performance liquid chromatographic method for the estimation of IBU and FAM in combined dosage form. The developed method was validated in accordance with FDA and ICH guidelines [31] and confirmed that the analytical procedure employed is suitable and reliable for its intended use.

EXPERIMENTAL

Materials

IBU and FAM were supplied as gratis sample from Manish Pharma Lab, Ahmedabad, India and Niralee Pharma, Ankleshwar, India, respectively. Extra pure

grade Methanol and Chloroform, AR grade Toluene and Ammonia were purchased from Sisco Research Laboratory, Mumbai, India. Nylon filter papers of 0.22 µm pore size were purchased from Millipore, Bangalore, India.

Analytical method

Experimental conditions

The chromatographic system comprised of an CAMAG HPTLC system equipped with a sample applicator Camag Linomat V (Semiautomatic Applicator) and Densitometric scanner for detection. The TLC plate was aluminum backed plates coated with 0.2-mm layers of silica gel 60 F254 and plate was developed in Camag Twin Trough Chamber (10x10 cm2, 20x10 cm2). The data were acquired and processed using Camag winCATS software v1.4.7. The mobile phase system consisted of Chloroform: Methanol: Toluene: Ammonia in the ratio of 3:1.2: 1: 0.1 5 % v/v/v/v. The mobile was run upto 70 mm and dried and detected in Densitometric scanner.

Chromatographic method development and optimization

Different mobile phase composition were tried to get better separation of FAM and IBU on TLC plate. Initially ethyl acetate and methanol were used in different in the ratio of 5:5, 6:4, and 5: 3 %v/v in which IBU travelled with mobile phase. Another mobile phase compositions like chloroform: toluene (5:3%v/v), chloroform: methanol (4:1%v/v), chloroform: methanol: toluene (4:1:1%v/v), chloroform: Methanol: toluene: ammonia (3:1:1:0.1%v/v) and chloroform: methanol: toluene: ammonia (3:1.2:1:0.1). Detection wavelength was selected by considering the λmax of FAM and IBU and relative height of the chromatographic peak and reproducibility of the detection was considered.

Preparation of stock solution and calibration standards

Accurately weighed quantity of 50 mg of FAM and 1500 mg of IBU were transferred to 100 mL volumetric flask containing 50 mL of diluent and dissolved with sonication. Final volume was made upto 100 mL with diluent to get Standard stock solution having known concentration of 0.5 mg/mL and 15 mg/mL of FAM and IBU respectively. Accurately measured 1 mL of standard stock solution was transferred to 100 mL volumetric flask. The final volume was made up to the mark with diluents to obtain a working standard solution having known concentration of 0.005mg/mL and 0.15mg/mL of FAM and IBU respectively.

Method Validation

Specificity and Selectivity

To determine the selectivity of the method Rf value of FAM and IBU from standard solution and sample solution were compared and peak shape was observed. Further, the absence of interference of excipients used in pharmaceutical preparation was demonstrated. The specificity was estimated from the purity of the peak obtained at specific Rf value of the analyte.

Linearity

From working standard solution 2, 3, 4, 5, 6, 7 and 8µLwere spotted on 8cm × 10cm HPTLC plate. Then spotted HPTLC plate was kept in pre saturated chamber and mobile phase was run up to 70 mm and scanned by scanner. Calibration curve was constructed by plotting mean peak areas of FAM and IBU against respective concentration. The experiment was repeated 5 times for linearity study.

Precision

The precision of the proposed method was assessed in terms of repeatability, intra-day precision, inter-day precision and reproducibility.

a) Reproducibility of the Instrument

To check sample applicator reproducibility six bands of standard solution (5 µL) containing 0.005mg/mL of FAM and 0.15mg/mL of IBU was spotted on a single TLC plate (80 mm × 100 mm). The 6 spots were analyzed on a single plate to observe repeatability and %RSD of the area for both the analyte were calculated.

Reproducibility of Densitometric scanner also measured by scanning of single track 6 times and %RSD of the area were calculated.

b) Intra-day precision

Intra-day precision were studied by spotting and analyzing 3 spots containing 15, 25 and 40 ng/spot of FAM and 300, 750 and 900ng/spot of IBU. The experiment was repeated 3 times in a single day at 2 hours interval. The SD and %RSD of the area was calculated to check the intra-day repeatability of the method.

c) Inter-day precision

To check the inter-day precision 3 different levels of concentration were spotted on a single TLC plate. The same experiment was repeated 3 times on 3 different days. The SD and %RSD was calculated of the area.

d) Reproducibility (with different analyst)

Reproducibility of the method was checked preparing all solution from starting and analyzing on same day by three different analysts and SD and %RSD was calculated from the results obtained.

Accuracy

The accuracy of the method was studied by recovery method in which preanalyzed test solution (18 ng/spot for FAM and 540 ng/spot for IBU) were spiked with standard solution in the concentration level of 80%, 100% and 120% (14.4, 18, and 21.6ng/spot for FAM and 432, 540 and 648 ng/spot for IBU) of the test solution which was given final amount per spot about 32.4, 36 and 39.6 of FAM and 972, 1080 and 1188 of IBU. The mixture was spotted and %recovery was calculated. The recovery study was repeated three times and average % recovery was calculated.

Limit of Detection and Limit of Quantitation

The limit of detection and quantitation were estimated based on the standard deviation of the intercept and slope. The standard deviation of y-intercept of the regression lines and slope were used for the calculation of the limit of detection and quantitation limit. The rquation to calculate LOD and LOQ were represented below,

$$LOD = \frac{3.3s}{s}$$

$$LOQ = \frac{10s}{s}$$

Robustness

The HPTLC method required strict experimental condition for the reproducible results than HPLC method. Different parameters like saturation time and mobile phase composition can affect the results obtained from the result. The capacity of different parameters to affect the results of the analysis was studied during robustness study. Different parameters like saturation time and mobile phase composition was deviated for some extent and effect of these changes was observed and %RSD of the area in obtained in different condition was calculated.

Stock Solution Stability

Two working standard solution were prepared and stored at laboratory condition and refrigerated temperature and analyzed continuously for 5 days in same experimental condition and % RSD was calculated.

Analysis of Excipients Influence on Developed Assay Method

The influence of the excipients used in the formulation on proposed analytical method was checked by preparing a physical mixture with the analyte and the chromatogram was compared with that of standard chromatogram obtained from analyte alone. The Rf value of both the analyte was compared on both the track and spectrum of both the analyte in both the track was compared for spectrum similarity.

Analysis of Marketed formulation

Sample solution (3 µL) was spotted on HPTLC plate in triplicate and area of both the analyte was measured and % assay was calculated from the below equations 1 and 2.

$$\%Assay\ of\ FAM = (Area - 124.78) - \frac{100}{700.90 \times 18}$$

$$\%Assay\ of\ IBU = (Area - 1.29) - (\frac{100}{285.79 - 540})$$

Calculations and Statistical Analysis

Standard regression curve analysis was computed using EXCEL® software (Microsoft Corporation, USA) without forcing through zero. Means and standard deviation were also calculated using the same software.

RESULTS AND DISCUSSION

Chromatographic Method Development and Optimization

The chromatographic conditions like mobile phase, detection wavelength, TLC plate size and saturation time were varied to get acceptable Rf value of both analytes. Different mobile phase compositions having ethyl acetate, methanol and toluene was tried but IBU travelled with the mobile phase. The mobile phase composition having different ratios of chloroform, methanol and toluene were showed separation and the Rf value of both analytes were adjusted with proportion of these three components. The mobile composition of Chloroform: methanol: toluene in the ratio of 3:1.2:1 was given Rf of 0.3 and 0.44 but band of FAM was broad and to reduce band broadening 0.1 % Ammonia was added and final optimized mobile phase was Chloroform: methanol: toluene: ammonia (3:1.2:1:0.1). The detection was done at 280 nm and saturation time 15 minutes. FAM and IBU gives Rf value of 0.34 and 0.46 in optimized chromatographic condition which is showed in figure 2. Selected wavelength of detection was 280 nm.

Method Validation

Specificity and Selectivity

The method was found to be selective because the Rf value of analytes obtained in test solution was similar to that of Rf value from standard solution as shown in Figure 3. The specificity of the method was confirmed by chromatographic peak purity was about 0.9999 for both the analyte. Spectrum obtained in standard mixture and formulation was also given correlation about 0.999963 and 0.999959 for FAM and IBU respectively and it was presented on Figure 4.

Linearity

Linearity range selected FAM was from 15-40 ng/spot covering amount per spot 15, 20, 25, 30, 35 and 40 ng and for IBU it was from 300-1200 ng/spot covering 300, 600, 750, 900 and 1200 ng/spot. Both analytes FAM and IBU were given linear response with co-relation coefficient of 0.9943 and 0.9953. The linearity range showed reproducibility and %RSD was found to be less than 2.0. The linearity data for FAM and IBU were shown in Table 1 and 2. The calibration curve and overlain chromatograms over linearity range is presented in figure 4 and 5.

Precision

a) Reproducibility of the Instrument

Reproducibility of the sample applicator and detection scanner was checked and data showed in Table 3. Both the component shown reproducibility within limit with %RSD less than 1.0 and could be used for the further validation.

b) Intra-day precision

The intra-day precision was checked spotting and evaluating 3 levels of concentration 3 times in a single day and the %RSD was found to be less than 2 and data

was presented in Table 4 and 5 for FAM and IBU, which confirms the intra-day repeatability of the method.

c) Inter-day precision

The %RSD for inter-day precision was found less than 2.0 and shows inter-day reproducibility within limit. The data for inter-day precision was presented in Table 6 and 7 for FAM and IBU respectively.

Accuracy

The data presenting recovery were shown in Table 8 and 9 for FAM and IBU respectively. The method had given the %recovery in the range of 97.45-102.11 for FAM and 97.85-103.42 for IBU.

Limit of Detection and Limit of Quantitation

The Limit of detection and quantitation was determined by equation as per ICH guideline Q2 (R1). The LOD was found to be 0.016 and 0.0003 ng/spot whereas, LOQ was found to be 0.049 and 0.00087 for FAM and IBU respectively.

Robustness

From the robustness data of the method presenting in Table 10, it can be concluded that, the method was less robust when saturation time changed and it should be kept 15 minutes to get reproducible results. The mobile phase volume was not affect majorly to the area of the spot which can be concluded from Table 11 but, it should be kept constant to 5.3 mL for better separation results and for plate size should also kept 8cm × 10 cm or of lower size.

Stock solution stability

The stock solution was stable at refrigerated temperature as well as at room temperature for at least for 5 days could be interpreted from Table 12.

Analysis of marketed formulation

The proposed method was successfully applied for the analysis of investigated substances in the commercially available tablet formulations of FAM and IBU. The % recovery was calculated from equation 1 and 2 for FAM and IBU respectively. The formulation assay results, expressed as a percentage of the label claim, are shown in Table. The formulation was assayed three times and % RSD was found less than 2. The low RSD values (<2%) confirmed the suitability of this method for routine analysis of FAM and IBU in pharmaceutical dosage forms. The % assay result of the formulation by proposed method was shown in Table 13.

Analysis of Excipients Influence on Developed Assay Method

The analytes and excipients were premixed and analyzed by proposed method to check absence of interference from excipients and % recovery of analytes from physical mixture. The chromatogram shows absence of interferences from the excipients and also given good recovery for both the analyte.

CONCLUSION

The proposed HPLC method for simultaneous estimation of FAM and IBU from combined dosage form was found to be precise, accurate and specific, as depicted by the statistical data of analysis. The developed method is rapid and completes a single run in relatively short time, i.e., five minutes. The proposed method enables rapid quantification and simultaneous analysis of both drugs from commercial formulations without any interference of excipients. It can, therefore, be concluded that the reported method could find practical application as an economical and rapid quality-control tool for simultaneous analysis of FAM and IBU from their combined dosage forms in both research and industrial quality-control laboratories.

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Figure 1 Chemical structures of Ibuprofen (IBU) and Famotidine (FAM)

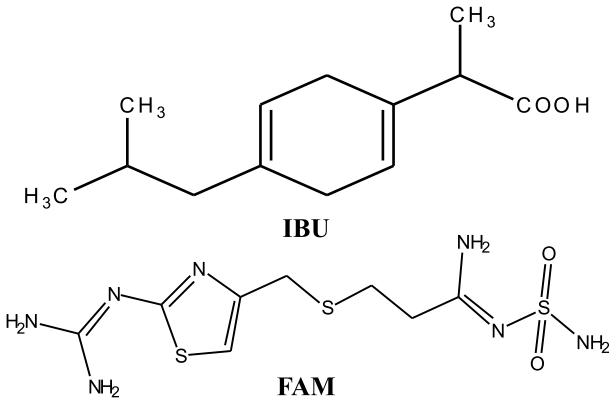

Figure 2 Chromatogram obtained from optimized mobile phase

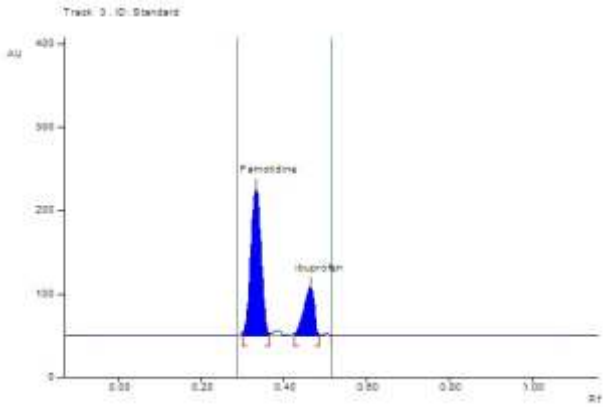

Figure 3 Comparisons of chromatogram obtained from (a) standard and (b) formulation sample.

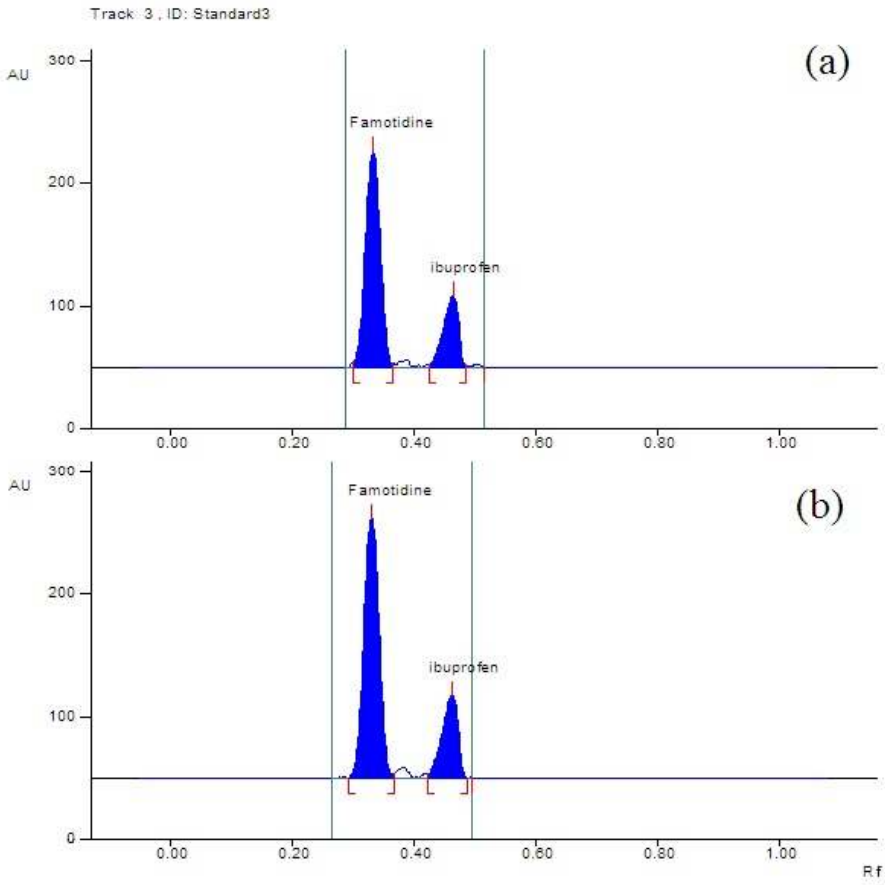

Figure 4 Spectrum similarity between standard and formulation chromatogram of FAM and IBU

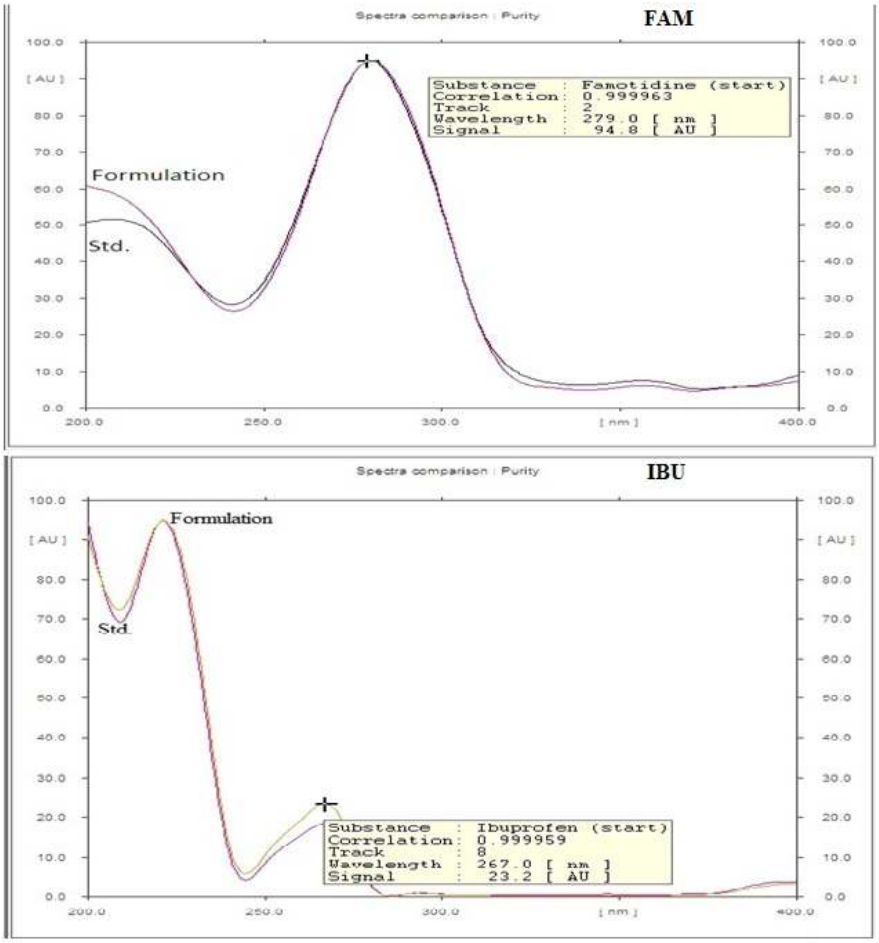

Figure 5 Calibration curve of (a) FAM and (b) IBU

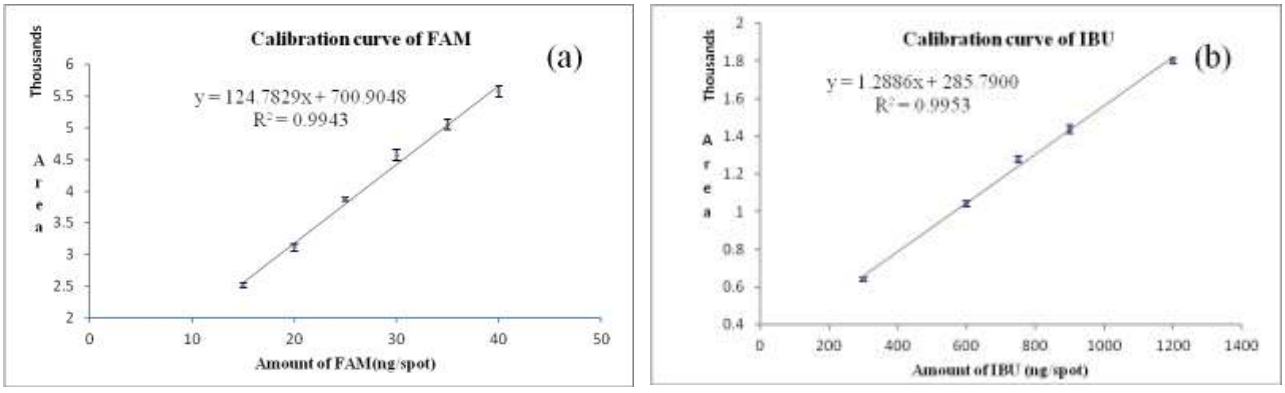

Figure 6 Overlay chromatograms of FAM and IBU over linearity range

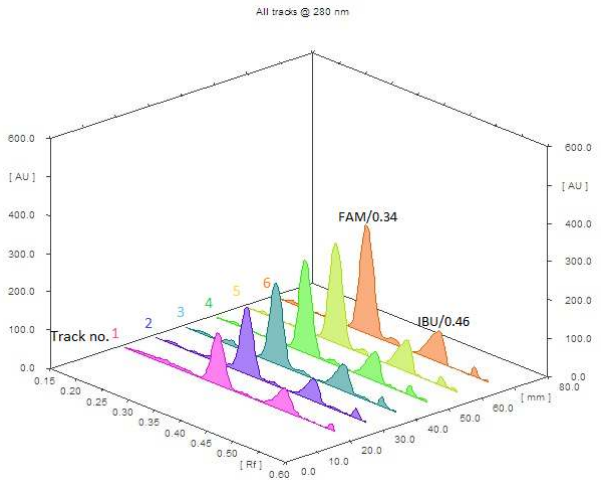

Table 1 Calibration data for FAM (n=5)

Sr.no.	Conc.(ng/spot)	Area ± S.D.	%RSD
1	15	2510 ± 41.91	1.67
2	20	3111 ± 57.30	1.84
3	25	3876 ± 35.23	0.91
4	30	4568 ± 86.98	1.9
5	35	5051 ± 81.93	1.62
6	40	5575 ± 92.34	1.65

Table 2 Calibration data for IBU (n=5)

Sr.no.	Conc.(ng/spot)	Area ± S.D.	%RSD
1	300	640 ± 9.52	1.49
2	600	1042 ± 14.75	1.42
3	750	1277 ± 18.29	1.43
4	900	1438 ± 20.04	1.39
5	1200	1798 ± 14.18	0.79

Table 3 Data Representing Reproducibility of the Instrument

Applicator Reproducibility	Area	Scanner reproducibility	Area
1	3799	1	3812
2	3783	2	3791
3	3802	3	3817
4	3812	4	3801
5	3783	5	3791
6	3714	6	3796
Mean	3782.167	Mean	3801.333
SD	35.25573	SD	10.96662
RSD	0.932157	RSD	0.288494

Table 4 Results of intra-day precision for FAM

Sr. no.	ng/spot	Intra-day Area ± SD	RSD
1	15	2502 ± 46.82	1.87
2	20	3089 ± 51.55	1.67
3	25	3865 ± 43.09	1.11
4	30	4508 ± 40.72	0.9
5	35	4996 ± 48.40	0.97
6	40	5511 ± 38.28	0.69

Table 5 Results of intra-day precision for IBU

Sr. no.	ng/spot	Intra-day Area ± SD	RSD
1	300	646 ± 1.01	1.01
2	600	1049 ± 7.10	0.67
3	750	1283 ± 14.57	1.14
4	900	1458 ± 22.03	1.51
5	1200	1790 ± 11.67	0.65

Table 6 Results of Inter-day precision for FAM

Sr. no.	ng/spot	Inter-day Area ± SD	RSD
1	15	2516 ± 33.50	1.33
2	20	3322 ± 64.42	1.99
3	25	3886 ± 22.37	0.57
4	30	4623 ± 78.08	1.69
5	35	5110 ± 64.45	1.26
6	40	5579 ± 85.29	1.53

Table 7 Results of Inter-day precision for IBU

Sr. no.	ng/spot	Inter-day Area ± SD	RSD
1	300	666 ± 7.00	1.05
2	600	1047 ± 10.15	0.96
3	750	1269 ± 1.40	1.4
4	900	1469 ± 1.44	1.44
5	1200	1812 ± 32.19	1.78

Table 8 Results of the Accuracy study of FAM

Amount of Sample (ng/spot)	Amount of drug added (ng/spot)	Total conc. (ng/spot)	Amt. recovered ± SD (ng/spot)	% Mean recovery ± S.D.	%RSD
18	0	18	18.13 ± 0.17	100.77 ± 0.94	0.93
18	14.4	32.4	32.78 ± 0.21	101.18 ± 0.65	0.64
18	18	36	36.04 ± 0.44	100.12 ± 1.25	1.25
18	21.6	39.6	39.53 ± 0.14	99.82 ± 0.35	0.35

Table 9 Results of the Accuracy study of IBU

Amount of Sample (ng/spot)	Amount of drug added (ng/spot)	Total conc. (ng/spot)	Amt. recovered ± SD (µg/mL)	% Mean Recovery ± S.D.	%RSD
540	0	540	540.53 ± 6.95	100.10 ± 1.20	1.20
540	432	972	976.36 ± 4.61	101.98 ± 0.99	0.98
540	540	1080	1080.01 ± 4.56	100.93 ± 0.84	0.85
540	648	1188	1191.31 ± 14.43	101.38 ± 2.32	1.21

Table 10 Robustness of the Method on Change in Saturation time

Saturation time	FAM		IBU	
(min.)	R_f	Area	R_f	Area
0	0.45	3659	0.53	1211
10	0.33	3701	0.41	1310
15	0.34	3863	0.46	1269

Table 11 Robustness of the Method on Change in Mobile phase volume

Total volume	FAM		IBU	
(mL)	R_f	Area	R_f	Area
5.3	0.34	3863	0.46	1269
10.6	0.33	3799	0.4	1231

Table 12 Stock solution stability at two different storage conditions

	Room Temp.		Refrigerated temp.	
Day	FAM	IBU	FAM	IBU
0	3870	1277	3835	1290
1	3861	1229	3832	1279
2	3819	1267	3810	1259
3	3856	1278	3799	1270
4	3840	1258	3841	1279
5	3863	1269	3791	1281
Mean	3851	1263	3818	1276
SD	18.83	18.19	20.82	10.61
RSD	0.49	1.44	0.55	0.83

Table 13 Assay Results of Commercial Tablets (n=3)

Analyte	Label claim (mg)	Amount found (mg)	% Assay	Mean % Assay	SD	RSD
FAM	26.6	26.51	99.66	99.50	0.96	0.96
	26.6	26.19	98.47			
	26.6	26.69	100.37			
IBU	800	795.41	99.43	99.66	1.09	1.09
	800	806.82	100.85			
	800	789.70	98.71			