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Original Article

Simultaneous Quantification of Famotidine and PABA by First Order Derivative Spectral Technique of UV Spectrophotometric from FMT-PABA Cocrystals

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ABSTRACT

Objectives: The investigation article intends is to build and validate a more convenient scientific approach to accurately simultaneously determine famotidine and para-amino benzoic acid from their cocrystals **Significance:** The zero-order spectra of both drugs demonstrated significant overlap between the two spectra curves; therefore, the simultaneous estimation of two drugs by zero-order spectra method was not feasible. Therefore, to overcome this and facilitate simultaneous analysis of both the therapeutic agent derivative spectrophotometry technique was employed; The sensitivity and selectivity of both the drug mixtures were enhanced by the derivative spectrophotometry technique. **Materials and Methods:** First-order derivative spectra were chosen for the quantitative analysis of FMT and PABA in cocrystals. Zero-crossing measurements (both calculated and plotted) were employed to select the wavelength for each drug to carry out quality control analysis. ICH guidelines were referred to validate the technique. **Results:** The wavelength 275 nm for FMT was selected referring to zero-crossing points (ΔA/Δγ of PABA is zero at 275 nm) and 285 nm was selected for PABA (ΔA/Δγ of FMT is zero at 285 nm) and no mutual intrusion was detected and these wavelengths were designated for estimation of these therapeutic agents. The linearity, accuracy, precision, and robustness of the method were validated by satisfying the statistical parameters. **Conclusion:** The technique established for the simultaneous assessment of drugs (FMT and PABA) from cocrystals by first-order derivative spectrophotometric technique was validated. The development of the analytical method was reliable, economical, convenient, and environmentally friendly.

Keywords: Zero-crossing, First-order derivative spectra, Simultaneous estimation.

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INTRODUCTION

Famotidine (FMT) is an antiulcer drug and the pKa value of the same, when determined by various methods viz solubility, spectrophotometric, and partitioning method has been reported as 6.98, 6.78, and 6.89 respectively. The intrinsic solubility of FMT has been indicated as 2.7mM by a pH-solubility study of the drug. Therefore, FMT has been classified under BCS class II specifying low aqueous solubility and high permeability.[1] It is a potent drug acting on the H2 receptor (Histamine H2 Antagonists).[2] Solubility restriction of the drug renders various pharmacokinetic parameter problems, which, in turn, affects the drug's action. The low aqueous solubility can be confronted either by adopting a reformulation approach or by physicochemical modification. Various reformulations, for instance, are nanoformulations, solid dispersions, cosolvency, use of hydrotropes, and many others.[3] Example of various physicochemical modifications is co-crystal formation,[4] salt form, and many more. Co-crystallization is a technique to increase the solubility of such therapeutic agents, as these generate a metastable supersaturated state, enhancing the solubility[5] and hence improving bioavailability with no alteration of the therapeutic effects. Hence, to improve the solubility of FMT, the cocrystal of FMT was prepared using PABA (Para Amino Benzoic Acid) as a coformer. This research article intends to build and validate an analytical technique aiming at quantification estimation of both FMT and PABA in the cocrystal of FMT-PABA. In the cocrystal of FMT-PABA, the first derivative spectrophotometric analytical method[6-8] is built and substantiated. The quantification of medicinal agents and coformer can also be determined by various sophisticated instruments viz high-performance liquid chromatography and ultrafast liquid chromatography but it requires expensive analytical grade solvents, which are toxic, flammable, and hazardous and cause pollution. Moreover, solvent consumption is too high with a general flow rate of almost 1.5 to 2 L of waste per day.[9-11] In this

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research article, we aim to employ the UV spectrophotometric technique which is a more convenient, economical, and reliable quantitative and qualitative analytical tool to estimate FMT in various pharmaceutical products. Cocrystals of FMT-PABA were acquired by the solvent evaporation method.

The sensitivity and selectivity of both the drug mixtures were enhanced by the derivative spectrophotometry technique. First-order derivative spectra were chosen for the quantitative analysis of FMT and PABA in cocrystals. Zero-crossing measurements (both calculated and plotted) were employed to select the wavelength for each drug to carry out quality control analysis. ICH guidelines were referred to validate the technique.

Chemical formula of FMT is 3-[[2-(diaminomethylideneamino)-1,3-thiazol-4-yl]methylsulfanyl]-*N*'-sulfamoylpropanimidamide, structure given in Figure 1a and PABA is para amino benzoic acid structure given in Figure 1b.

MATERIALS AND METHODS

FMT and PABA and Highly purified analytical grade solvents and reagents, such as methanol, were purchased from CDH Laboratories, New Delhi, India. Distilled water was obtained from the distillation Unit of NIET (Pharmacy Institute).

Preparation of Cocrystals

Co-crystals of Famotidine (FMT) with Para-Amino-Benzoic Acid (PABA) were prepared by a solvent evaporation method. Accurately weighed drugs and co-formers of 1:1 molar ratio/ equimolar ratio were dissolved in methanol separately. Famotidine was dissolved in 30ml of methanol and the temperature was maintained at 40°C while stirring coformers were dissolved in 20 mL of methanol separately with continuous stirring. The two solutions were mixed and stirred for 10-15 min. till a clear solution is obtained, further followed by filtration through filter paper (Whatman). The beaker holding the solution was shielded with aluminium foil. The foil was pierced into 4-5 fine holes on the foil and kept in the beaker for 48-72 hr. to facilitate the evaporation of the solvent from the solution.[12,13] The solid co-crystals were obtained after the complete evaporation of the organic solvent and stored in a desiccator.

Stock Solution Preparation

10 mg of FMT was added to 100 mL of 0.1 N HCl and sonicated for 15 min, followed by dilutions to achieve the to obtain a concentration ranging from 5-25 µg/mL. Similarly, a succession of concentrations extending from 5-25 µg/mL of PABA was prepared.

The cocrystal solution was prepared by dissolving 20 mg of cocrystal in 100 mL of 0.1N HCl and sonicated for 15 min. followed by further dilution to get a concentration of 20 µg/mL of cocrystal.

Selection of wavelengths 250 to 350 nm, considering 0.1 N HCl as the blank solution on UV visible double beam (4) with 2 nm and 60 nm/min, the bandwidth and a scanning speed, respectively. All drug (each at 10 µg/mL) spectra were obtained separately to get a zero-order spectrum. Further, the drug spectra were converted into the first-order derivative spectra from the programs given in the UV instrumentation and using Microsoft Excel for both drugs. Both the first-order spectra derivatives of FMT and PABA were overlapped to select the wavelength of FMT where PABA shows zero crossings (means zero absorbance) and the wavelength of PABA where FMT shows zero crossings.

Method Validation

Conferring to guidelines (ICH) procedure validation is performed for selectivity, accuracy, linearity, precision, the limit of detection, and the limit of quantification and robustness.[14-18]

Selectivity

Selectivity was estimated by the investigation of FMT and PABA at both the wavelength 275 nm (zero crossings for PABA) and 285 nm (zero crossings for FMT) selected, respectively.

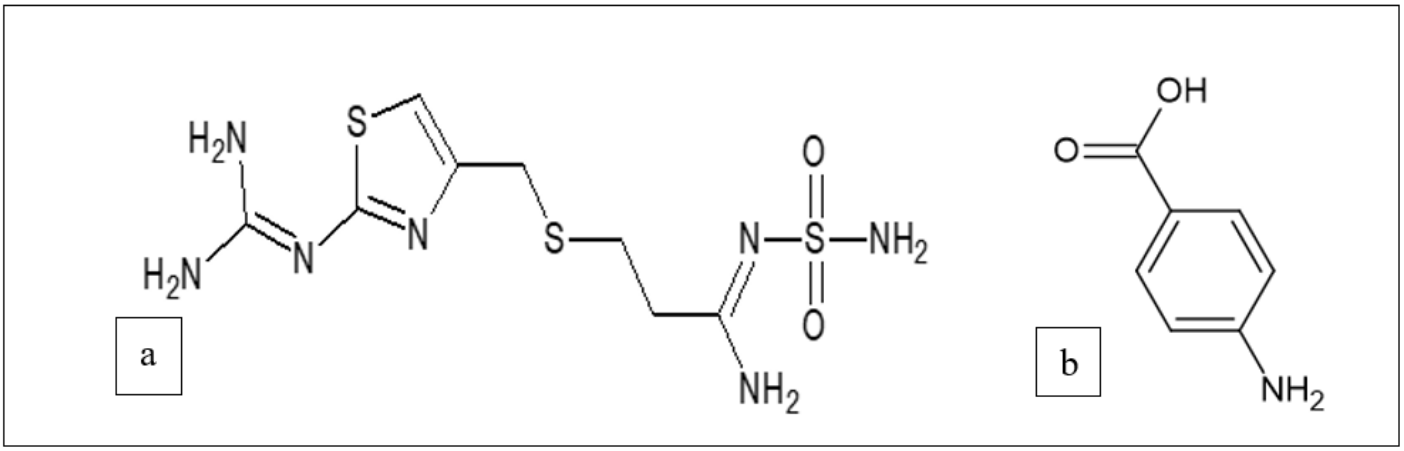

Figure 1: a) Structure of Famotidine and b) Structure of Para amino benzoic acid.

Linearity

Linearity was resolute by plotting between concentration versus first-order derivative of absorbance values (at 275 nm for FMT and at 285 nm for PABA), for the mixture of both FMT and PABA comprising 5 concentrations of two assigned drugs, in the range of 5-25 µg/mL (5, 10, 15, 20 and 25 µg/mL). The experiment was carried out in triplicate. Least square regression analysis was employed to assess linearity, and this was further confirmed by Analysis of Variance (ANOVA). From the calibration curve using equations 1 and 2 Limits of Detection (LOD) and Limit of Quantification (LOQ) were estimated using the equation 1 and 2, SD_b= standard deviation of the y-intercept line of regression.

$$LOD=(3.3*SD_b)/\text{slope of the line of regression} \quad (1)$$

$$LOQ=(10*SD_b)/\text{slope of the line of regression} \quad (2)$$

Accuracy

The calculation of the extent of accuracy (n=3) was established by the percent recovery of the given amount of drugs (FMT and PABA) totalled to the cocrystal. The cocrystal samples were further spiked with three concentrations from both the pure drugs (2,4,6 µg/mL) standard solutions. The concentration of cocrystal taken was 20 µg/mL. As cocrystals were formed in the ratio of 1:1 molar concentration of FMT and PABA, therefore 20 µg contains 14.3 µg of FMT and 5.7 µg of PABA.

Precision

The intraday (three phases in a particular day) and interday precision (for three days) evaluation was done for the cocrystal at the consistent concentration of 20 µg/mL (contains 14.3 µg of FMT and 5.7 µg of PABA). Relative Standard Deviation (RSD) was employed to statistically express precision. All experiments were carried out in triplicate.

Robustness

Robustness indicates the ability to withstand the deliberated small variations in the method parameters viz small variations in the calibration or effect of small factors like temperature of the system, pH of the system, period of sonication, scanning speed, and many more. Robustness was analyzed using a split-plot regular two-level design, this fractional factorial design was selected to estimate the main and combined effect of the various factors encountered on a daily basis. Therefore, in this study, three factors at two levels each specified as pH at 7 and pH at 3.5, scanning speed (40 nm/min and 60 nm/min), and sonication time (10 min and 20 min). Sixteen experiments were conducted for the assay of FMT and PABA. The outcomes were evaluated by t-test and the level of significance was determined to find if $t_{calculated} > t_{critical}$

RESULTS AND DISCUSSION

We intend to use this method to estimate the release kinetics and solubility of the cocrystals of FMT-PABA *in vitro* simulating the gastric environment, therefore 0.1 N HCl was selected as the solvent. The overlap of the zero-order UV spectra of FMT and PABA (Figure 2) and illustrations of the wavelength at maximum absorption in 0.1 N HCl at 290 nm and 275 nm, respectively. From the graph, it is visible that there is a significant overlap between the two spectra curves it is not probable to simultaneously

Figure 2: Zero-order Spectra of FMT and PABA.

Table 1: Linearity parameter of FMT and PABA by least square regression method.

Parameters	FMT	PABA
Concentration range	5 to 25 µg/mL	5 to 25 µg/mL
Correlation (r^2)	0.9908	0.9974
Equation of line of regression	y=0.003x-0.0019	y=-0.0029x-0.0109
LOD	0.55 µg/mL	0.68 µg/mL
LOQ	1.66 µg/mL	2.06 µg/mL
ANOVA	Significant at $p<0.001$	Significant at $p<0.001$

Table 2: Using the first-order derivative spectrophotometric method for Recovery test for FMT and PABA.

Analyte Sample	Sample concentration (µg/mL)	Standard concentration (µg/mL)	Theoretical value (µg/mL)	Actual value found[a]±SD (µg/mL)	% Recovery	%RSD
FMT	14.3	2	16.3	16.4±0.26	100.61	1.61
FMT	14.3	4	18.4	18.3±0.13	99.45	0.55
FMT	14.3	6	20.3	19.9±.06	98.02	0.29
PABA	5.7	2	7.7	7.8±0.12	101.29	1.28
PABA	5.7	4	9.7	9.5±0.17	97.93	1.82
PABA	5.7	6	11.7	11.8±0.1	100.85	0.85

Pure samples of FMT and PABA are considered here as the standard concentration whereas the cocrystal was referred as the sample concentration.a=mean of three determinates, SD=Standard Deviation, RSD=Relative Standard Deviation.

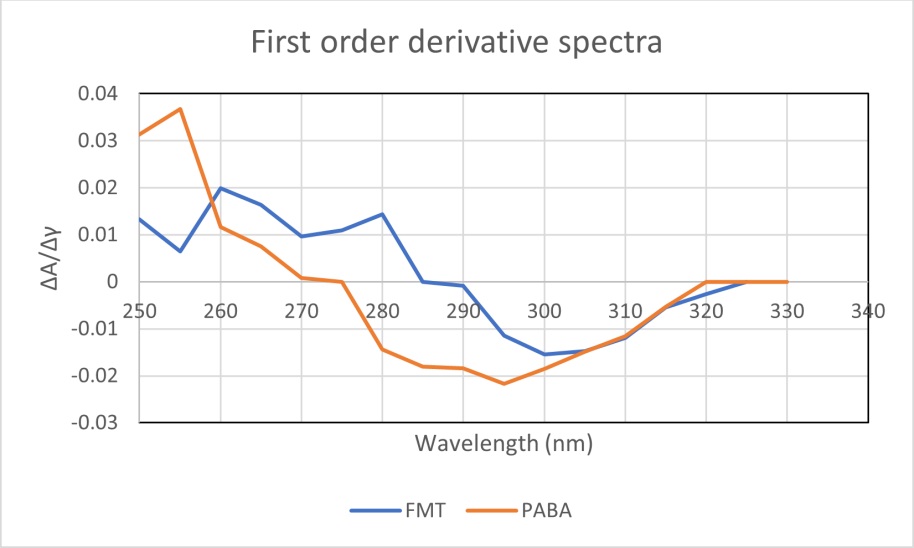

Figure 3: First-order Derivative Spectra of FMT and PABA.

estimate two drugs by the zero-order spectra method. Therefore, to overcome this and facilitate simultaneous analysis of both the therapeutic agent derivative spectrophotometry technique was employed, Figure 3 displays that the zero-crossing points were ΔA/Δγ of PABA is zero at 275 nm, hence this wavelength was selected for FMT and 285 nm for PABA as ΔA/Δγ of FMT is zero at 285 nm and no mutual intrusion was detected and these wavelengths were designated for estimation of these therapeutic agents.

Method Validation

The results of various parameters confirming the validation of the technique adapted are explained in this section.

Selectivity

The selectivity studies indicated no interference between the medicated agents as no cross-interference was detected throughout the investigation. FMT and PABA presented zero ΔA/Δγ at 285 and 275 nm, correspondingly.

Linearity

Demonstrating that the facility of the technique, having the ability to deliver outcomes that is directly proportional to the concentration of the analyte in the sample was expressed in terms of linearity. The concentration of the analyte in the sample is directly proportional to $\Delta A/\Delta\gamma$ is evident in the result as given in Table 1.

Accuracy

Accuracy articulates the closeness of the theoretical values and the experimental value. The actual value referred to in the Table 2 denotes the experimental value which was in accordance with the theoretical value as depicted in Table 2. The % recovery of both drugs was within the desired limit ranging from 101.29 to 97.93% for three added levels for each drug. The %RSD value for each reading was found to be <2%, which further confers the validity of the recovery study.

Precision

Precision is a parameter that addresses the nearness of agreement between the different magnitudes attained of the same analyte on several sampling under the given conditions. The intraday and inter day precision as depicted in Table 3 was observed that %RSD value is within the standard range (<%), therefore confirming the precision of procedure designed.

Robustness

It estimates the ability of a method to withstand by the minute but delibrated changes in the experimental parameters, it points out the reliability of the method on repeated use. The analysis report was obtained as depicted in Table 4, split-plot regular two-level design has been employed (to characterize a scientifically large number of factors using a lesser number of experiments) and the factors influencing were studied and found that the design facilitates the screening of many factors using various comparative parameter. The result shows that the mean assay values are between 100.67 and 99.56 for FMT and 100.86 and 99.54 for PABA. As the determined t values were lesser than the critical t values (α=0.05), it indicated that variation in factors had insignificant effect on the outcomes. Thus, statistically proving the robustness of the system.

Table 3: Intra-day and inter-day precision analysis.

Intra-day Precision										
Analyte Sample	Sample concentration (µg/mL)	Slot 1			Slot 2			Slot 3		
		Conc. found	Mean Conc	%RSD	Conc. found	Mean Conc	%RSD	Conc. found	Mean Conc	%RSD
FMT	14.3	14.22	14.16±0.21	1.49	14.4	14.26±0.14	0.98	14.56	14.43±0.17	1.18
FMT	14.3	14.33			14.27			14.24		
FMT	14.3	13.92			14.12			14.50		
PABA	5.7	5.62	5.72±0.09	1.59	5.64	5.68±0.05	0.90	5.74	5.72±0.03	0.44
PABA	5.7	5.73			5.67			5.72		
PABA	5.7	5.8			5.74			5.69		
Inter-day Precision										
	Sample concentration (µg/mL)	Diurnal 1			Diurnal 2			Diurnal 3		
		Conc. found	Mean Conc	%RSD	Conc. found	Mean Conc	%RSD	Conc. found	Mean Conc	%RSD
FMT	14.3	14.22	14.16±0.21	1.49	14.32	14.28±0.03	0.23	14.33	14.21±0.23	1.65
FMT	14.3	14.53			14.26			14.36		
FMT	14.3	13.92			14.27			13.94		
PABA	5.7	5.62	5.72±0.09	1.59	5.82	5.77±0.06	1.06	5.67	5.74±0.07	1.22
PABA	5.7	5.73			5.70			5.81		
PABA	5.7	5.8			5.78			5.74		

Slot represents various timings on the same day.

Table 4: Split-plot regular two-level design used to demonstrate robustness of the analytical method developed.

Runs	Factor A	Factor B	Factor C	% Assay of FMT	% Assay of PABA
1	-1.0	-1.0	-1.0	100.15	100.43
2	1.0	1.0	-1.0	100.2	99.90
3	1.0	-1.0	-1.0	99.85	100.23
4	-1.0	1.0	-1.0	100.45	100.70
5	-1.0	-1.0	1.0	100.34	100.34
6	1.0	-1.0	1.0	100.67	100.54
7	-1.0	1.0	1.0	100.23	100.69
8	1.0	1.0	1.0	100.49	99.87
9	-1.0	-1.0	-1.0	100.51	100.53
10	1.0	1.0	-1.0	100.43	100.48
11	1.0	-1.0	-1.0	99.56	100.54
12	-1.0	1.0	-1.0	99.77	100.64
13	-1.0	-1.0	1.0	100.28	100.86
14	1.0	-1.0	1.0	100.63	99.54
15	-1.0	1.0	1.0	100.56	99.94
16	1.0	1.0	1.0	100.47	100.48
t_{cal} (FMT)	7.33E-29	4.67E-12	6.76E-20		
t_{cal} (PABA)	7.76E-29	4.58E-12	6.73E-20		
$t_{critical}$	2.13145				

% Assay: quantitative determination of FMT and PABA. Factor A represent pH (+) high factor level: for pH 7 and (–) low factor level: for pH 3.5. Factor B represent scanning speed (+) high factor level: for 60 nm/min and (–) low factor level: for 40 nm/min. Factor C represent sonication time: (+) high factor level: for 20 min and (–) low factor level 10 min.

CONCLUSION

The technique built for the simultaneous assessment of drugs (FMT and PABA) from cocrystals by first-order derivative spectrophotometric technique was validated. FMT and PABA presented zero ΔA/Δγ at 285 and 275 nm, correspondingly. The development of the analytical method was reliable, economical, convenient, and environment friendly. The split-plot regular two-level design was used to demonstrate the statistically robustness of the analytical method developed. By various validation methods, it was proved that the technique is selective, linear, accurate robust, and precise, facilitating the determination of the analyte in a more scientific approach.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FMT: Famotidine; **PABA:** Para-amino benzoic acid; **H2 receptor:** Histamine Receptor; **BCS:** Biopharmaceutics Classification System; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **SD:** Standard deviation of the y-intercept line of regression; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification.

SUMMARY

The objective of the article is to build and validate a more convenient scientific approach to accurately simultaneously determine famotidine and para-amino benzoic acid from their cocrystals using first-order derivative spectrophotometry technique. The zero-order spectra of both drugs demonstrated significant overlap between the two spectra curves; therefore, the simultaneous estimation of two drugs by zero-order spectra method was not feasible. The wavelength 275 nm for FMT was selected referring to zero-crossing points (ΔA/Δγ of PABA is zero at 275 nm) and 285 nm was selected for PABA (ΔA/Δγ of FMT is zero at 285 nm) and no mutual intrusion was detected and these wavelengths were designated for estimation of these therapeutic agents. The linearity, accuracy, precision, and robustness of the method were validated by satisfying the statistical parameters. The technique established for the simultaneous assessment of

drugs (FMT and PABA) from cocrystals by first-order derivative spectrophotometric technique was validated.

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