DEVELOPMENT AND VALIDATION OF A UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF FAMOTIDINE IN SUSPENSIONS

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Abstract

The reference pharmacopeias provide HPLC methods for determination of Famotidine a potent H_2- receptor antagonist in Pharmaceutical preparations, which are costly and unaffordable for most pharmaceutical laboratories and research students. Keeping in view the socio-economic conditions of our country there is a need to develop an alternate technique, so that the research studies become easier and cheaper. The present study is on the development and validation studies of a UV spectrophotometric method for the quantitative determination of Famotidine as active and in formulation *i.e* Suspensions. The method includes extraction of an aqueous layer using organic solvent Diethyl ether and measurement of absorbance at 265 nm. Different aspects of validation were taken into consideration such as Accuracy, Precision, Linearity, Specificity, Limit of detection and Limit of quantification. The method is found to be specific with no interference of excepients and is suitable over a range of 10-50µg /mL of Famotidine. The mean % recovery was found to be 99.32%-100.17 % (n=6). The intra and inter day RSDs (n=6) were 0.45%-0.57%. The method is accurate, cost effective and fast.

Introduction

Famotidine is a H_2 receptor antagonist available for treatment of ulcers (duodenal and gastric).It blocks H_2- receptors located on parietal cells and inhibit gastric acid secretions (Goodman and Gillman, 1996). Famotidine is given by mouth or parenterally by IV route (Sweetman, 2002). It has excellent tolerability profile and lowest incidence of side effects (Howden and Tytgat, 1996).

Two simple spectrophotometric methods were developed for determination of trazodone HCl, famotidine and diltiazem in pure and pharmaceutical preparation. The apparent molar absorptivity for method A was 1.06×10^5, 2.9×10^4, 1.2×10^4 and for method B was 9.4×10^4 and 1.6×10^4, respectively (Ayad *et al.*, 2003). Another Spectrophotometric method was developed for determination of Famotidine, based on reaction of Famotidine with Cobalt (II) to form a complex with an absorption maximum at 319 nm The method was applicable over a concentration range of 10-60 µg/mL and the recovery was quantitative (Kelani *et al.*, 2002).

In a sensitive study, low concentrations of histamine H_2-receptor antagonist were affected across a water plug, with separation taking place in binary buffer comprising ethylene glycol and NaH_2PO_4 (pH 5) and detection at 214 nm liquid- liquid extraction with ethyl acetate-isopropanol provide clean extracts. The calibration curves were linear over a concentration range of 0.1-3.0 µg/ mL of famotidine. Mean recoveries were >82 % while RSDs and REs (relative errors) were all < 13%.The method was sensitive with a detection limit of 10ng/ mL Famotidine (Ko *et al.*, 2001). A spectrophotometric method was described for the determination of Famotidine in tablet or bulk powder. In the assay a methanolic extract of famotidine were mixed with 0.2% chloranil in toluene methanol(2:3:2 mL), methanolic 0.2% 2,3 dichloro-5,6-dicyano-6-nitrophenol (1 mL) diluted to 10 mL with methanol ,and set aside at 28^{o}C for 30 min. Absorption was measured at 458,460 and 425nm respectively. The ranges for three reagents were 50-500, 40-450 and 10-100 µg/ mL of the compound in final solution, respectively (Kamath *et al*., 1992)

A simple spectrophotometric method for determination of Famotidine in commercial dosage forms was based on the oxidation of drug with alkaline potassium permanganate. Absorbance was measured at 610 nm. At ranges 2-10 and 1-8 µg / mL the calibration

curves were linear, using initial rate and fixed time methods respectively. (Rahman and Kashif, 2003). A spectrophotometric method for determination of famotidine in pharmaceutical preparations based on formation of a 1:1 ion pair complex with both bromocresol green (BCG) and bromothymol blue (BTB), and its subsequent extraction with an organic solvent was developed (Abu-Zuhri *et al*., 1999).

Two titrametric and two spectrophotometric methods were described for the assay of famotidine in tablet using N- bromosuccinimide. (NBS) The first method was direct in which Famotidine was titrated directly with NBS in HCl medium using methyl orange as indicator .Other methods were indirect in which unreacted NBS

was determined after complete reaction between Famotidine and NBS In spectrophotmetric methods Beer's law obeyed over the concentration ranges of 0.75-6 µg/ mL and 0.3-3 µg/ mL. (Zenita and Basavaiah, 2011) A sensitive HPLC method was developed and validated for determination of Famotidine and its impurities in pharmaceuticals using porous graphite column. Method was linear over a range of 1.5 -1000µg/ mL. All recoveries were greater than 98 %. (Helali and Monser, 2008) A reverse phase Liquid chromatography method was developed for simultaneous determination of Famotidine and ibuprofen. (Ahirrao and Pawar, 2013).

A precise RP-HPLC method for simultaneous estimation of Famotidine and Domeperidone in Pharmaceutical dosage was developed in which linearity was found to be 2.5-50 µg/mL while percent content of Famotidine was 98.56±0.83. (Aruna *et al*., 2013). A RP-HPLC method was developed to estimate amount of Famotidine in bulk and formulations using potassium dihydrogen phosphate buffer of pH 7 and acetonitrile in ratio of 40:60 v/v as mo- bile phase at wavelength 297 range was 20-60 µg/mL. Mean recovery was 99.8%. (Reddy *et al*., 2012) A method for Simultaneous determination of metformin, cimetidine, famotidine and ranitidine in human serum and dosage formulation using HPLC with UV detection at 229nm was developed. (Arayne *et al.,* 2010).A selective and precise HPTLC method for determination of Paracetamol, Diclofenac potassium and Famotidine in bulk and tablet formulation was developed and validated. (Khatal *et al*., 2010)

Materials and Methods

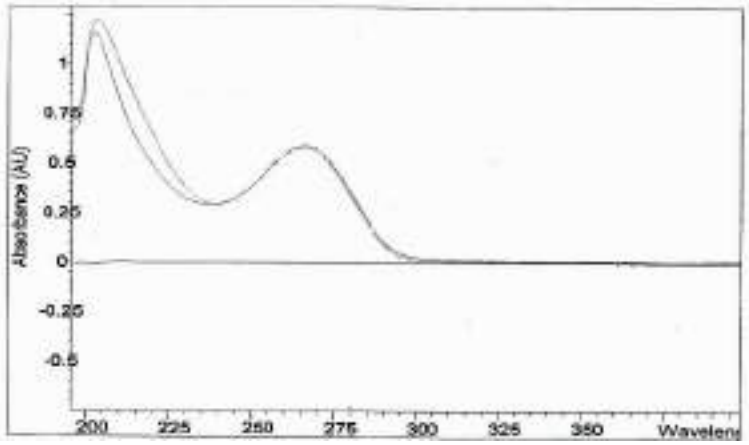
Famotidine was provided by Saffron Pharmaceuticals Pvt. Limited, Faisalabad, Pakistan. Other chemicals as HCl, Diethylether were of analytical grade (Merck, Germany).

Preparation of standard: Accurately weighed quantity equivalent to 25 mg of Famotidine working standard was dissolved in 50mL volumetric flask and made up to the mark with 0.1N HCl. The resulting standard solution was vortexed and sonicated. 2 mL of this standard solution was taken in 50 mL volumetric flask and diluted up to the mark with 0.1 N HCl, later vortexed and sonicated. Absorbance of his solution was measured at maximum wavelength 265nm using 0.1 N HCl as blank.

Preparation of sample: In a 50mL beaker, suspension equivalent to 25 mg of Famotidine was taken, 30mL of 0.1N HCl was added and stirred until the suspension was completely dissolved in 0.1N HCl, then transferred into 50mL flask and volume was made up to the mark with 0.1 N HCl. The resulting solution was centrifuged for 15 min at 2500 RPM. The clear solution (supernatant) was taken in extraction flask and 40mL of Diethylether was added. Organic layer was discarded and extraction was repeated three times with 40mL ether. Finally the aqueous layer was collected, refluxed at 60^{o}C for 5 minutes and then cooled. 2mL of this cooled solution was diluted up to 50 mL with 0.1 N HCl and its absorbance was measured at maximum wavelength 265 nm, using 0.1 N HCl as blank.

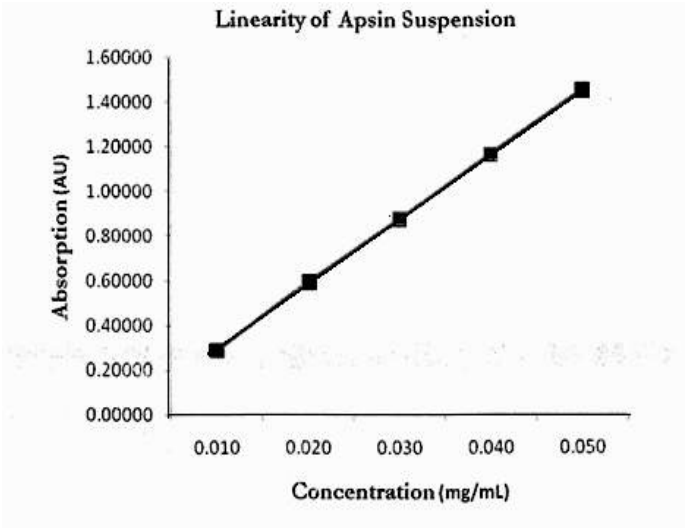
Parameters of validation and results

Specificity: To check the specificity the reference material famotidine was tested in presence of excepients of Apsin 10mg/5mL suspension with the same ratio as use in Apsin 10 mg/5 mL suspensions.



Representative UV spectra of Famotidine against the excipients of Apsin 10mg/5mL Suspension.

Linearity: Sample was prepared as described Quantitation of analyte depends upon if obeying beer's law and is linear over a concentration range. Five dilutions were prepared for linearity. Statistical evaluation of linearity of famotidine is given in Table I linearity.



Accuracy: Accuracy was assayed using six samples prepared at 100% of the test concentration as per procedure described in section of preparation of sample Summary of statistical analysis of accuracy is given in TABLE II.

Range: The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has the suitable level of precision, accuracy and linearity.

Precision: According to ICH guidelines, there are two categories of precision: repeatability and intermediate precision.

Repeatability: Repeatability was assayed by nine determinations .Three samples were prepared at 80%, 100% and 120% of the test concentrations procedure given in section of preparation of sample. Each sample preparation was analyzed three times .Statistical analysis is given in Table III.

Intermediate precision: Typical variations studied including days, analysts and equipments etc. A minimum of six analyses were performed over a minimum of two occasions.

Six samples were prepared at 100% of the test concentration as per procedure given in section preparation of sample. Each sample was analyzed and recovery of all determinations was reported.

- Same analysis was performed by different analysts on the same day and instrument Statistical analysis is given in Table IV.
- Same analysis was performed on different days by same instruments and same analyst. Statistical analysis is given in Table V.

Limit of Quantification (LOQ): It is used particularly for the determination of impurities and degradation product.

Limit of Detection (LOD): It is determined by the analysis of sample with known concentration of the analyte and by establishing the minimum level at which the analyte can be reliably detected. Table VI**.**

Results and Discussion

The spectrum obtained of famotidine reference standard and placebo indicated that the method is specific only for famotidine and the excepients of 10mg/5mL suspension have no effect as they show no activity at 265 nm which is λ_{max} of Famotidine as shown in the spectrum**.** Table I for linearity clearly indicates that 10µg is the lowest and 50 µg is the highest limit of Famotidine in a sample at which the analytical procedure has the suitable level of precision, accuracy and linearity .This is the range of analytical method.

Repeatability was accessed using nine determinations having a maximum RME of 0.1681 which is very rare value 0.29% RSD showed that there is good degree of agreement among individual results when the method is applied to multiple samplings of homogenous sample. The data, for different analyst on same day, was assessed using twelve determinants having %RSD of 0.54 and 0.45 showed that there is good degree of agreement among individual results .The calculated value of F is 1.438 is less than the tabulated value corresponding to five percent probability which is 5.05.This presents that there is no significant difference between the precisions, at the 5% level. The data, for same analyst on different days, was assessed using twelve

determinations having % RSD of 0.45 and 0.57 showed that there is good degree of agreement among individual results. The calculated value of F is 1.603 is less than the tabulated value corresponding to five percent probability which is 5.05.This presents that there is no significant difference between the precisions, at the 5% level.

Results derived from Table I indicates that 10µg is lowest amount of Famotidine in a sample that can be quantified with this method. Below this concentration, a result varies within replicates.

The entire data was reviewed statistically and the detection limit was calculated which is 0.00001 mg/mL or 0.01293µg/mL that is the lowest amount of Famotidine in a sample that can be detected.

Table I. Statistical evaluation of the linear part of calibration dependence of famotidine.

Compound	Range (mg/mL)	Intercept	Slope	Coefficient of Correlation (*r*)	% RSD
Famotidine	0.010-0.050	0.00285	28.983	0.99993	0.0830

Table II. Accuracy of Famotidine in Apsin 10mg/5mL suspension.

Compound	**Amount of drug added (mg)**	**Amount of drug found (mg)**	**(SD)[a]**	**% Recovery**	**% RSD**	**Relative mean error (RME)**	**Confidence limits[b]**
Famotidine	24.92	24.74	0.0922	99.32	0.37	0.1521	24.666- 24.817

[a]n=6

[b]Confidence limits at p=0.05 and five degrees of freedom.

Table III. Evaluation of precision of Famotidine in Apsin 10mg/5mL suspension.

Conc.	**Amount of drug added (mg)**	**Amount of drug Found (mg)**	**(SD)[a]**	**% Recovery**	**% RSD**	**Relative mean error(RME)**	**Confidence limits[b]**
80%	22.72	22.75	0.02646	100.17	0.11%	0.0671	22.705-22.795
100%	24.92	24.84	0.07234	99.71	0.29%	0.1681	24.721-24.965
120%	27.84	27.85	0.06083	100.02	0.22%	0.1261	27.747-27.953

[a]n = 3

[b]Confidence limits at p=0.05 and two degrees of freedom.

Table IV. Evaluation of Intermediate precision of Famotidine by Different analyst on same day(intra day) and instrument.

Amount of drug added (mg)	**Amount of Drug Found (mg)**	**(SD)[a]**	**% Recovery**	**% RSD**	**Relative mean error(RME)**	**Confidence limits[b]**
24.92	24.82	0.13387	99.63	0.54%	0.2202	24.710-24.930
24.97	24.87	0.11165	99.59	0.45%	0.1833	24.775-24.959

[a]n = 6

[b]Confidence limits at p=0.05 and five degrees of freedom

Table V. Evaluation of Intermediate precision of Famotidine by same analyst on Different days (inter days).

Amount of drug added (mg)	Amount of drug Found (mg)	(SD)[a]	% Recovery	% RSD	Relative mean error(RME)	Confidence limits[b]
25.06	24.87	0.11267	99.27	0.45%	0.1850	24.776-24.961
24.86	24.89	0.14264	100.16	0.57%	0.2339	24.776-25.011
n = 6						
Confidence limits at p=0.05 and five degrees of freedom						

Table VI. Evaluation of the limit of detection of Famotidine.

Compound	Conc.	r	Intercept	Slope	LOD (*mg/mL)*
Famotidine	0.0050	1	-0.00006	29.5104	0.00001 Or 0.01293

Conclusion

The method was validated by using all the parameters and on the basis of results obtained it is concluded that the UV spectrophotometric analysis of Famotidine in Apsin 10mg/5mL suspension has been successfully done . The results obtained agreed with those obtained by official methods.

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