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Research Article

Area Under Curve Method Development And Validation Of Fenspiride Hydrocholride In Pharmaceutical Dosage Form

Ashish Balasaheb Jadhav[1*], Vishal Bharat Babar[2], Priti Rajendra Kale[3]

Abstract

Quick, inexpensive, accurate, precise, and repeatable, the suggested method may be used for routine quality control examination of this medication in pharmaceutical dose forms. The approach is ideal for determining the presence of Fenspiride HCl in pharmaceuticals because the analytical techniques were precise, exact, and specific. Six injections of a solution containing analyte at 100% of test concentration will be used as part of system suitability testing to ascertain the accuracy and precision of the system. Plate count, tailing factors, resolution, and repeatability (percent RSD of retention time, peak area, and height for six injections) are the parameters that will be determined. The AUC findings show the instrumental response for the calibration curve's reference standard. Fenspiride HCl was found to be linear over the concentration range of 5–25 µg /ml. According to ICH criteria, the developed methods were validated in terms of linearity, accuracy, precision, and sensitivity. Validation was then used to demonstrate the applicability of these methods for quantitative chemical determination.

Keywords: Area under curve, Fenspiride HCl, quantitative investigation, linearity, accuracy, precision.

INTRODUCTION

The employment of an analytical strategy followed by a process of creating evidence that offers a high level of certainty is a crucial phase in the discovery of new drugs. Despite the drug's great potency, it won't be able to be sold because there isn't an approved analytical procedure. This protects the quality and security of the medication[1]. This study suggests multiple validation criteria in line with various regulatory agencies, as well as quantitative analytical development methodologies for evaluating drug stability. It is mainly involved in the identification or detection of compounds and quality measurements of the substance present in bulk and pharmaceutical preparations[2-6]. It's a process by which specific analytical are developed for drugs evaluation of new or novel pharmaceutical products from the stage of the in process to the finished product and a mini validation to be done before starting the analysis of routine sample when there are no definitive methods or techniques. The most frequent types of analytical processes, according to the International Conference on Harmonization (ICH) ARE identification tests, quantitative testing of the active moiety in API or drug product samples or other drug product selected components, quantitative tests for impurity content, and limits tests for the control of impurities[7-10].
The requirements of the analytical method need to develop the analytical figures of merit such as linearity, selectivity, range, accuracy, precision, detection limits etc., shall be defined. There are no previous suitable methods for analyzing the analyte to be examined. The second-order derivative is distinguished by a negative band with a minimum at the same wavelength as the maximum on the zero-order band[11-14]. This derivative also demonstrates two positive satellite bands on either side of the main band. The fourth derivative shows a significant positive band with a maximum at the

[1*]Research Scholar, Sunrise University, Alwar, Rajasthan, India. ashishjadhav973@gmail.com
[2]Dattakala College of Pharmacy, Swami-Chincholi (Bhigwan), Maharashtra, India.
[3]Research Scholar, Sunrise University, Alwar, Rajasthan, India.

same wavelength as the zero order band's maximum. Even-order derivatives have a negative or positive band with a minimum or maximum at the same wavelength as the absorbance band's maximum.

DRUG PROFILE

Fenspiride is chemically known as: 8-(2-di phenyl ethyl)-1-oxa-3,8-diazospiro[4,5] decan-2- one hydrochloride. Its chemical formula and molar mass were $C_{15}H_{20}N_2O_2$.HCl and 296.79g/mol. Fenspiride is an oxazolidinone spiro compound used as a drug in the treatment of certain respiratory diseases. It is approved for use in Russia for the treatment of acute and chronic inflammatory diseases of ENT organs and the respiratory tract (like rhinopharyngitis, laryngitis, tracheobronchitis, otitis and sinusitis), as well as for maintenance treatment of asthma. Fenspiride (INN, brand names Eurespal, Pneumorel and others) is an oxazolidinone spiro compound used as a drug in the treatment of certain respiratory diseases[15-18]. The pharmacotherapeutic classification is antitussives. In Russia it was approved for the treatment of acute and chronic inflammatory diseases of ENT organs (ear, nose, throat) and the respiratory tract (like rhinopharyngitis, laryngitis, tracheobronchitis, otitis and sinusitis), for maintenance treatment of asthma. Russia, Romania, France and other European countries withdrew fenspiride-based drugs from the market due to the risk of QT prolongation and torsades de pointes. Fenspiride is known to have activity as an alpha-1 blocker. It possesses anti-broncho constrictor and anti –Inflammatory properties stipulated by interaction of several related mechanisms: It blocks H_1 histamine receptors and makes spasmolytic effect of bronchus unstriated muscles. It makes anti-inflammatory action leading to reduction of various pro-inflammatory factor production (Cytokines, TNF-a, Arachidonic acid derivatives, Prostaglandins, leukotrienes, thromboxanes, free radicals) some of them also make bronchoconstrictor actions. It also inhibits α-adreno receptor stimulation of which causes increased production of bronchial secretions[19-22].

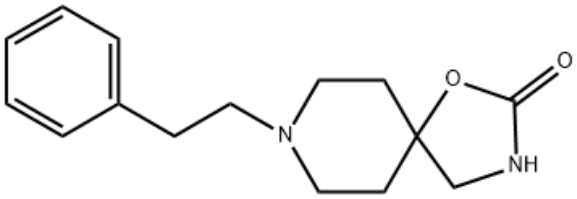

Fig-1: Fenspiride HCl

MATERIALS AND METHODS

Chemicals and reagents: the pure sample of Fenspiride HCl and Distilled water was used for whole experiment.

Tablet formulations: Brand B- Pneumoral80mg. Each film coated tablet contains Fenspiride HCl-80mg.

Instruments: A Shimadzu 1800 UV (Shimadzu Japan) spectrophotometer with 1 cm matched quartz cells was used for estimation.

Selection of media: Main criteria of media selection and stability, i.e. drug should be soluble as well as stable for sufficient time in selected media. Preliminary drug solubility studies: Fenspiride HCl was weighed and its solubility was tested in 50 mL water, 0.1 acetronitrile, and 50 mL methanol. The drug was shown to be water soluble and only partly soluble in methanol. As a result, water was chosen as a diluent, and the medication was shown to be stable in water. Distilled water was used as the analytical medium for this study[23].

Preparation of standard stock solution:

The standard stock solution was prepared by transferring 50 mg Fenspiride HCl in to a 50 ml volumetric flask. 50 ml Distilled water was put in to this volumetric flask and dissolved. To make a

solution containing 1000 µg/ml Fenspiride HCl, the volume was brought up to the mark with distilled water. 5 mL of this solution was transferred to a 50 mL volumetric flask, and the volume was adjusted to the mark using distilled water, yielding a solution containing 100µg/mL of Fenspiride HCl[24].

Preparation of calibration curve:

To make dilutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml, pippete out 5, 10, 15, 20, and 25 ml from a standard stock solution of Fenspiride HCl (100g/ml). At the λmax of Fenspiride HCl, these solutions were scanned in the 200-230nm region against distilled water as a blank, and then a calibration curve was plotted as absorbance vs. concentration to confirm the linear connection between absorbance and concentration of Fenspiride HCl.

Table-1: Standard calibration for Area Under Curve

S. No.	Concentration (µg/ml)	α	β	α+β
1	10	0.0023	1.4565	1.4542
2	15	0.1046	2.3286	2.4332
3	20	0.2152	3.0894	3.3026
4	25	0.1587	4.088	4.2467
5	30	0.6926	4.8335	5.526

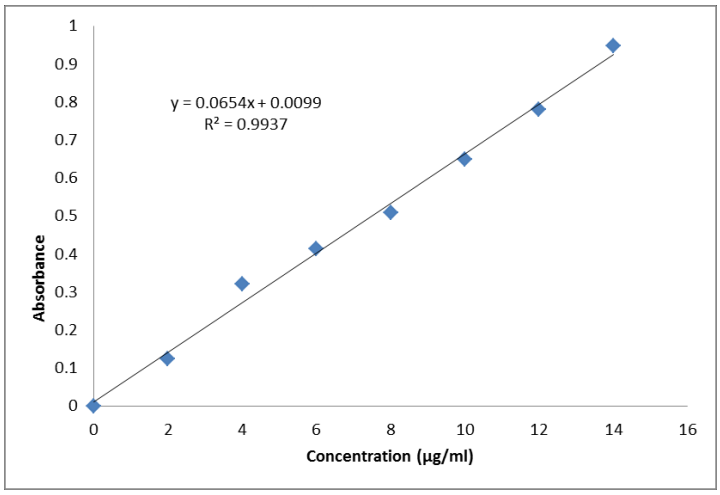

Fig-2: Standard calibration curve of Area under Curve for Fenspiride HCl

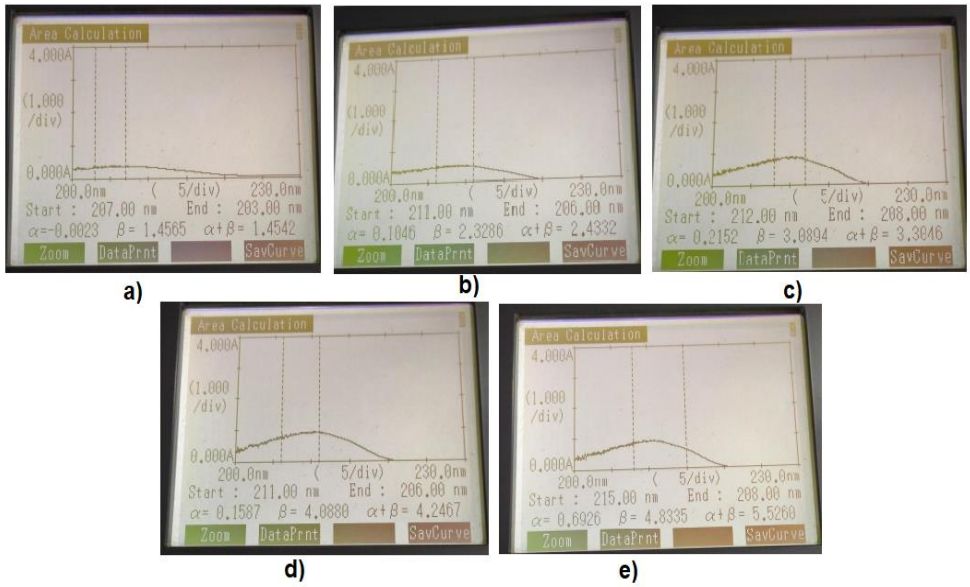

Fig-3: Instrumental response Area under Curve of Fenspiride HCl at a) 10µg/ml, b) 20µg/ml, c) 30µg/ml, d) 40µg/ml, and e) 50µg/ml

Validation of proposed method:

The commercialized a brand of Fenspiride HCl tablet strips was brought in for formulation analysis. Calculate the tablet's total weight. Then, take 10 tablet weights individually. It is necessary to smash the tablets. Prepare the 100 g/ml stock solution after calculating the weight to be taken. Take the absorbance at 228 nm[25].

Table-2: Assay of Fenspiride HCl in tablet formulation

Amount Taken (mg/tab)	Amount found (mg/tab)	Amount found (%)
80	79.98	99.92
80	80.04	100.45
80	79.08	99.15
80	79.95	99.66
80	80.33	100.72
	Mean	99.98
	SD	0.6252
	CV	0.0063

Linearity

To make dilutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml, pipette out 5, 10, 15, 20, and 25 ml from a standard stock solution of Fenspiride HCl (100g/ml). At the λmax of Fenspiride HCl, these solutions were scanned in the 200-290nm region against distilled water as a blank, and then a calibration curve was plotted as absorbance *vs* concentration to confirm the linear connection between absorbance and concentration of Fenspiride HCl.

Table-3: Linearity for AUC

S. No.	Concentration (µg/ml)	α	β	α+β
1	10	0.0023	1.4565	1.4542
2	15	0.1046	2.3286	2.4332
3	20	0.2152	3.0894	3.3026
4	25	0.1587	4.088	4.2467
5	30	0.6926	4.8335	5.526

Precision

Repeatability (intraday precision) and interday precision are two types of precision studies. Three times on the same day and three times on three different days, the same concentration of Fenspiride HCl (10µg/ml) was estimated. The precision study's findings were expressed as a percent relative standard deviation.

Table-4: Results of Intraday Precision Study

Concentration (µg/ml)	AUC	AUC	AUC	Average RSD
	MORNING	AFTERNOON	EVENING	
15	2.3221	2.1444	2.2654	2.4396
15	2.4133	2.4535	2.4987	2.4553
15	2.4332	2.4511	2.4111	2.4318
15	2.3564	2.2987	2.3516	2.3355
15	2.4143	2.4661	2.2555	2.3786
% RSD	0.04172	0.1254	0.0913	0.08614

Table-5: Results of Interday Precision Study

Concentration (µg/ml)	AUC	AUC	AUC	Average RSD
	DAY 1	DAY 2	DAY 3	
15	2.3654	2.3145	2.2136	2.2978
15	2.1146	2.4234	2.3145	2.2842
15	2.4121	2.4331	2.4631	2.4361
15	2.3154	2.4563	2.2689	2.3469
15	2.3624	2.3241	2.3874	2.3580
% RSD	0.1048	0.0588	0.0882	0.08392

Accuracy:

Recovery experiments were used to assess the method's accuracy. The recovery experiments were carried out by putting known amounts of substances into tablets. The recovery was carried out at three levels: 80, 100, and 120 percent of the standard Fenspiride HCl concentration. The recovery samples were prepared using the previously described procedure. For each level of recovery, three samples were prepared. The solutions were examined, and the percentage recoveries were determined using a formula[26].

$$\% \text{ Recovery} = \frac{\text{Observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \text{ x } 100$$

Table-6: Result of accuracy study

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	80	64	143.46	99.45	99.645	0.496	0.005
80	80	64	143.8	99.277			
80	80	64	144.7	100.21			
100	80	80	180.3	100.651	99.916	0.0684	0.0068
100	80	80	179.29	99.298			
100	80	80	179.78	99.8			
120	80	96	216.65	100.522	99.706	0.708	0.0071
120	80	96	215.77	99.342			
120	80	96	215.59	99.254			

LOD and LOQ:

The lowest concentration of analyte that can be detected is defined as the limit of detection (LOD), whereas the lowest concentration of analyte that can be quantitated is defined as the limit of quantitation. With the necessary precision and linearity the following formulas can be used to compute LOD and LOQ.

LOQ = 10 * r/ S, LOD = 3.3* r/ S

Where r is the standard deviation of the regression line's y-intercept and S is the slope of calibration curve.

Table-7: Results of LOD and LOQ

Drug	LOD(µg/ml)	LOQ (µg/ml)
Fenspiride HCl	2.1909	6.6391

RESULTS AND DISCUSSION

The derivative spectra were obtained at N=4 using a Shimadzu 1800 spectronic UV- Visible spectrophotometer, and the standard solutions of Fenspiride in distilled water (10g/ml each) were subjected to a scan 200 nm to 230 nm at first order. 228nm was determined to be the maximum

wavelength. At 228 nm, the Fenspiride HCl calibration curve was found to be linear. Beer's law was seen to be obeyed in the concentration range of 5-25 g/ml. The technique was validated using ICH guidelines for a variety of parameters such as specificity, linearity, accuracy, precision-repeatability, and the results were found to be satisfactory, with lower standard deviation and coefficient of variation values within acceptable limits for FenspirideHcl in its combined synthetic mixtures and combined dosage forms, such as marketed tablet formulation. The linearity for the first derivative method was obtained within the concentration range of 5 to 25µg/ml. the results were seen directly proportional to the analyte concentration.

The regression parameter of calibration curve obtained by first derivative method was in linearity range of 5 to 25µg/ml and value obtained is regression co-efficient (r2)=1.000. Accuracy test for Fenspiride HCl was studied by preparing standard solution of different conc 80,100,120% the percentage recoveries obtained were satisfactory. For the quantitation of Fenspiride HCl, the method outlined provides precise and accurate findings. Satisfactory recovery experiments at various degrees of confidence were also used to determine the method's accuracy. Intermediate precision investigations were done by several analysts, and the findings were determined to be adequate, indicating that the procedure was reproducible. The scheme was not sensitive to changes in method parameters since the results obtained were reproducible in varied temperature settings utilized at the time of identifying these drug compounds with very minimal variations under the circumstances employed.

The percentage standard deviation results indicate that the suggested method provides adequate Fenspiride HCl variation. The suggested technique's standard deviation percentages are within acceptable ranges for Fenspiride HCl, demonstrating the technique's ability to remain unaffected by minute and deliberate changes in system constraints and ensuring its consistency in regular routine use. The accuracy of the standard addition method was evaluated using three replicate measurements of three different solutions containing 80, 100, and 120 percent Fenspiride HCl. For three different concentrations, the average percent recovery was found. The higher outcomes showed the accuracy of the suggested UV spectrophotometric method for measuring Fenspiride HCl in pharmaceutical dosage form. This report compiles the results of recovery research. The limit of detection was determined to bel, while the limit of quantification was found. The results were within specification, indicating that the proposed technique for estimating Fenspiride HCl in bulk and tablet dose form was sensitive. The LOD and LOQ results are presented in (Table-7).

CONCLUSION

It may be concluded from the examination of the offered method's outcomes that a well- developed methodology ought to be easy to validate. Develop a strategy with the intention of quickly analyzing. There is no way for figuring out and validating Fenspiride HCl of First Derivative and Area under Curve in Pharmaceutical dose forms, according to a review of the literature on this medication. The assay's analytical methods were specific, exact, and accurate, making it a system suitable for figuring out the presence of Fenspiride HCl in medicines. The proposed method may be utilized for routine quality control analysis of this medicine in pharmaceutical dosage forms because it is easy, affordable, accurate, precise, and repeatable. To create a technique and implement a validation process to demonstrate that the strategy can be applied for the intended purpose. The results obtained for the first derivative follow Beer's Law. The AUC results indicate the instrumental response for the standard calibration curve.

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