Development and Validation of First-Order Derivative Spectrophotometry for Determination of Fenspiride Hydrochloride in Pharmaceutical Dosage Form

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ABSTRACT

For the quantitative detection of Fenspiride HCl in pharmaceutical products, simple, sensitive, focussed, accurate, and cost-effective UV-Spectrophotometric approaches were developed. Identifying the first-derivative spectrophotometric methods that were used to determine the Fenspiride HCl λ-max at 288 nm is the first step in the process. Distilled water was used as the diluent solvent throughout the procedure. The linearity of the substance was proven over the concentration range of 5–25 µg/ml. The second technique included the area under the Curve computation. 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: Fenspiride HCl, quantitative investigation, first derivative, area under curve.

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INTRODUCTION

Quantitative analysis seeks to establish the amount of a given element or compound in a sample. It is mainly involved in the identification or detection of compounds and qualitymeasurements of the substance present in bulk and pharmaceutical preparations[1-4]. 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. Analytical techniques are used to determine a drug's identification, purity, physical properties, and potency. Methods are created to help with drug testing against specifications throughout production and quality release activities, as well as long-term stability investigations. Methods may also be used to assist research on drug safety and characterization, as well as drug performance evaluations. The following are the most frequent types of analytical processes, according to the International Conference on Harmonization (ICH): identification tests,

quantitative testing of the active moiety in API or drug product samples or other drug product selected component(s), quantitative tests for impurity content, and limits tests for the control of impurities[5-9]. The derivative spectra of a spectrum expressed as absorbance (A) as a function of wavelength (λ) are: Zero order: A=f(λ), First order: dA/dλ=f'(λ), Second order: d2A/dλ2=f'(λ), third order: d3A/dλ3=f'"(λ).

Derivative spectra are usually more complicated than zero-order begins and ends at zero, passing through 0 at the same wavelength as the absorbance band's maximum. This Principles and applications of UV-visible spectroscopy derivative feature a positive and negative band with maximum and minimum values at the same wavelengths as the absorbance band inflection points. This bipolar function is seen in all odd-order derivatives[10-13]. 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. 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 same wavelength as the zero order bands 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[14-17].

Fenspiride HCl (INN, brand names Eurespal, Pneumorel, and others) is anoxazolidinone spiro compound used to treat respiratory disorders. The pharmacotherapeutic classification is antitussives. It was authorized in Russia for the treatment of acute and chronic inflammatory disorders of the ENT organs (ear, nose, throat) and the respiratory tract (such as rhinopharyngitis, laryngitis, tracheobronchitis, otitis, and sinusitis), as well as asthma maintenance therapy[18-20]. Due to the danger of QT prolongation and torsades de pointes, Russia, Romania, France, and other European nations pulled fenspiride-based medicines off the market. Fenspiride is a non-steroidal anti-inflammatory (NSAID). Medication that works by blocking histamine H1-receptors. It inhibits histamine-induced contraction of the trachea of the guniea pig but not histamine-induced inotropy of the isolated guniea pig heart[21-23]. It also inhibits PDE4 (phosphodiesterase 4), PDE5 (phosphodiesterase 5), and PDE3 (phosphodiesterase 3).

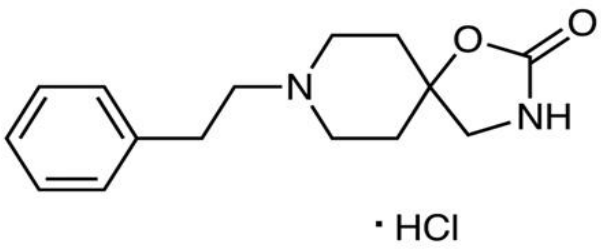

Fig-1: Fenspiride HCl structure

DRUG PROFILE

Molecular formula: $C_{15}H_{21}ClN_2O_2$
Molecular weight:296.79 g/mol
Chemical name: 8-(2-phenylethyl)-1-oxa-3,8-diazaspiro[4.5]decan-2-one HCl
Synonyms: Descaspiride
Category: Antitussive

Description: white crystalline powder
Melting point: 235-238℃
Solubility: Freely soluble in water and methanol, slightly in acetronitrile
U.V Absorbance: 200-230nm
Purity: ≥95%

Any pharmaceutical industry's main objective is to consistently manufacture goods with the required characteristics and quality at a reasonable cost. For the research, development, and assessment of medications in the pharmaceutical formulation, a technique must be developed. The ultimate focus of this research report was to evaluate the development and validation of the drug in pharmaceutical products procedure. To develop new alternative analytical method for spectrophotometric method for determination of Fenspiride HCl by first derivative. Development of AUC for UV-spectrometric for the quantitative estimation of Fensipiride HCl by first derivative. Validation of First Order Derivatives and area under curve for Fenspiride HCl. Application for the marketed products of Fenspiride HCl[24].

MATERIALS AND METHODS[25]

Standard drugs and their suppliers: Tablet formulation, Brand B- Pneumore 80mg. Each film coated tablet contains Fenspiride HCl 80mg.

Chemicals and reagents: The pure form of Fenspiride HCl and Distilled water

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.

Preparation of standard stock solution: The standard stock solution was prepared by transferring 50 mg Fenspiride HCl into a 50 ml volumetric flask. 50 ml Distilled water was put in to this volumetric flask and dissolved. To make a solution containing 1000 g/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 100µ g/mL of Fenspiride HCl.

Determination of λ max: To obtain a solution with a concentration of 10µg/ml, 5 ml of standard stock solution of Fenspiride HCl was transferred to a 50 ml volumetric flask and the volume was adjusted to the mark with the same solvent. The solution was scanned in the UV range 200-400 nm, with the maximum wavelengths being 288 nm, respectively. Fenspiride HCDl spectra were recorded.

Study of Beer-Lambert's Law: As Beer's Law states that the concentration of chemical

solution is directlyproportional to its absorption of light.

From the standard stock solution of Fenspiride HCl different volumes 2.5, 5, 7.5, 10, 12.5 ml were transferred to five separate 50 ml volumetric flask and volume was brought up to the mark with Distilled water to produce concentrations of 5,10, 15, 20, 25 µg/ml and a calibration curve was produced.

Table-1: Standard Calibration for Fenspiride HCl at 288 nm.

S. No.	Concentration of Fenspiride HCl (µg/ml)	Absorbance at 288nm
1	5	0.090
2	10	0.100
3	15	0.110
4	20	0.120
5	25	0.130

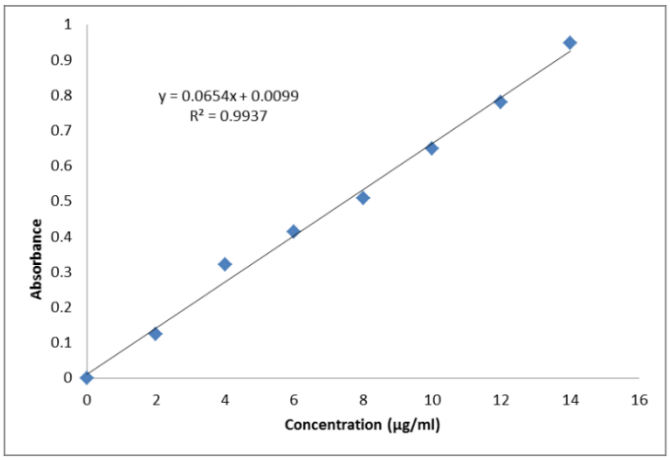

Fig-2: Calibration curve of Fenspiride HCl at 288 nm.

Dilutions of 5,10,15,20 and 25 µg/ml are prepared by using Distilled water as a solvent. The above concentrations are scanned in UV Spectrophotometer for first order derivative at N=4. Optical and regression parameters of the calibration curve obtained by first derivative method. The optical Parameters of the calibration curves are given in Table.

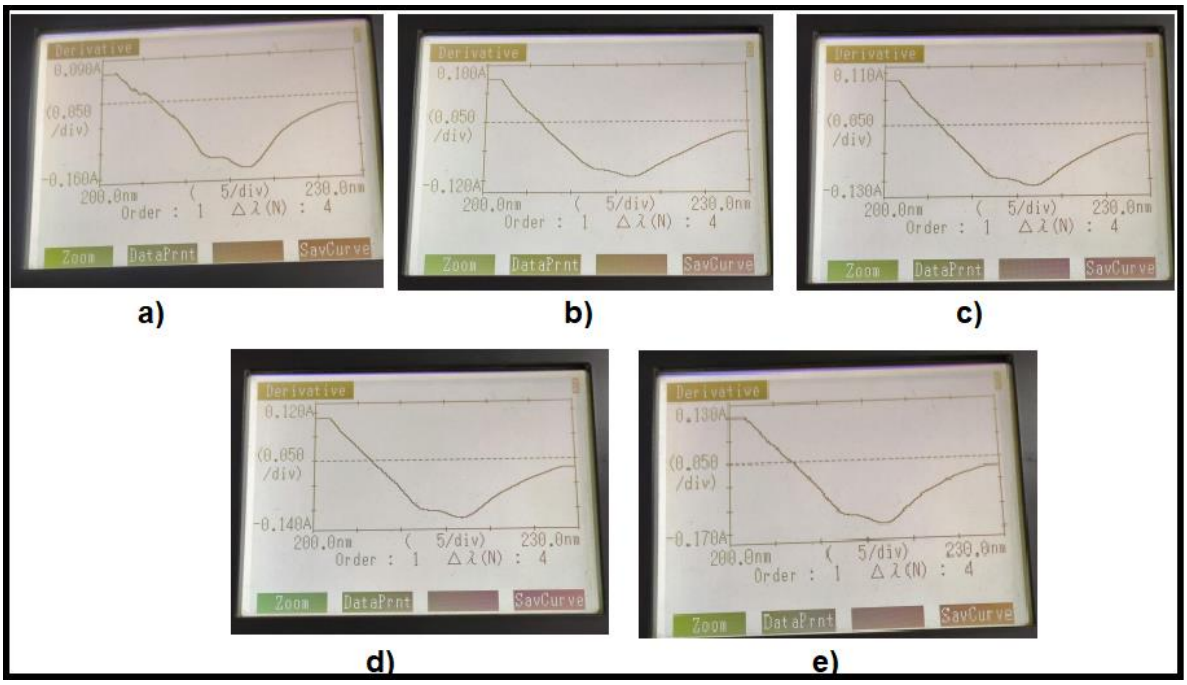

Fig-3: First derivative spectrum of Fenspiride Hcl at a) conc. 5 µg/ml b) conc. 10 µg/ml c) conc. 15 µg/ml d) conc. 20 µg/ml e) conc. 25 µg/ml

Table.2: optical Parameters of the calibration curves

Parameters	Fenspiride HCl
Linearity range (µg/ml)	5-25
Slope	0.002
Intercept	0
Regression coefficient(r^2)	1.000

Determination of Fenspiride HCl in bulk: The suggested technique for estimating Fenspiride HCl in commercialized pharmaceutical formulations was first tested for drug estimate in a typical bulk sample to establish if it was feasible. Fenspiride HCl was accurately weighed and transferred to a 50 ml volumetric flask, where it was dissolved in Distilled water by vigorous shaking and the volume was corrected to the mark using the same solvent. To reach the concentration of 100 g/ml, a suitable aliquot of 5 ml was transferred to a 50 ml volumetric flask and the volume was adjusted to mark with the same solvent to produce a concentration of 10 g/ml. The solution's absorbance was measured at 228 nm against a blank, and the results are presented.

Validation of proposed method[26]: Fenspiride HCl tablet strips from brand were brought in for investigation of commercial formulation. Calculate the tablet's total weight. Then 10 tablet weights were taken individually. Break the pill in half. Prepare the 100 g/ml stock solution after calculating the weight to be taken. Take the absorbance at 288 nm after preparing the 10 µg/ml solution. Table shows the results.

Table.3: Assay of Fenspiride HCl in tablet formulation (Brand A)

Amount taken(mg/tab)	Amount found(mg/tab)	Amount found(%)
80	79.98	99.10
80	80.11	100.11
80	79.88	99.90
80	79.95	99.96
80	80.21	100.81
Mean		99.97
SD		0.6097
CV		0.0061

RESULTS AND DISCUSSION

Accuracy (Recovery Test):

Recovery studies were used to test the method's accuracy. The recovery tests were carried out by introducing known quantities of a tablet. The recovery was done at three different concentrations: 80, 100, and 120 % of the Fenspiride HCl standard concentration. The above-mentioned technique was used to prepare the recovery samples. For each recovery level, three samples were produced. The percentage recoveries were calculated using formula after the solutions were analyzed[27].

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

Table.4: Results of accuracy parameter of Fenspiride HCl (Brand A)

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			

Precision:

Assay of method precision was evaluated by carrying out three independent assays of test sample of Fenspiride HCl. The intermediate precision of the method was also evaluated using four different analysts, systems in the same laboratory. The Assay values obtained by four analysts were summarized in Table.

Table.5: Determination of Precision of Fenspiride HCl for the firstderivative method.

Sample Number	Assay of Fenspiride HCl as % of labeled amount			
	Analyst- 1	**Analyst-2**	**Analyst-3**	**Analyst-4**
1	99.45	99.88	99.44	99.77
2	99.85	99.55	99.5	100.82
3	99.75	99.88	100.76	99.88
4	100.02	91.53	99.96	99.66
5	99.28	100.79	99.88	99.33
6	99.39	99.71	99.62	99.87
Mean	99.62	99.89	99.86	99.88
S.D.	0.2923	0.4664	0.4866	0.4993
CV	0.0029	0.0047	0.0049	0.005

The derivative spectra were obtained at N=4 using a Shimadzu 1800 UV-Visible spectrophotometer, and the standard solutions of Fenspiride in distilled water (10g/ml each) were subjected to a scan 200 nm to 290 nm at first order. 288nm was determined to be the maximum wavelength. At 288 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 Fenspiride HCl in its combined synthetic mixtures and combined dosage forms, such as marketed tablet formulation. Linearity is the ability of the method to elicit results that re directly proportional to analyte concentration within the given range. 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[28].

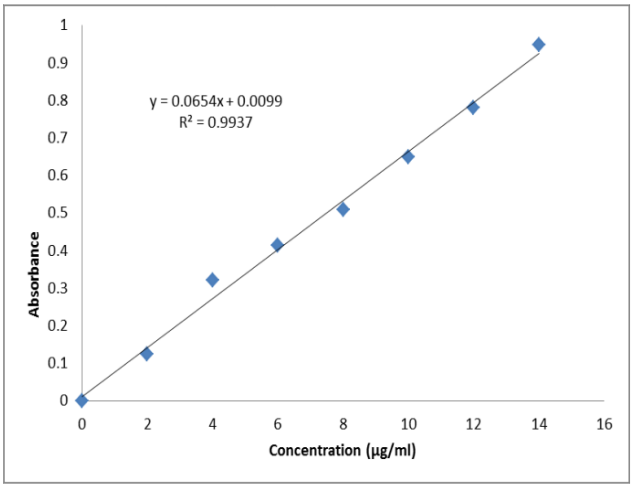

Fig-4: Calibration curve of Fenspiride HCl

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. 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.

Once the most acceptable approach for the analysis of pharmaceutical formulations has been chosen, the analysis should be done at least twice, preferably three times. Through experimental data acquired, which is a reflection of the analytical sample to be determined, a simple computation is turned into information. As with any physic- chemical measurement, the results acquired from actual experiments will always belinked with a level of uncertainty. As a result, determining the extent of this uncertainty is always required in order to transform the data into meaningful analytical findings that can be presented. As a result, it is important to validate the established approach and demonstrate the technique's capacity to correctly analyse the material under study. Standards must safeguard pharmaceutical components and formulations from either accidental contamination or purposeful contamination. Based on the aforementioned, the goal of this study was to develop quantitative analytical techniques for the quantitative estimation of some selected combinations or single drugs present in their synthetic bulk mixtures and multi-component formulations for cost-effective routine analysis such as dissolution studies, drug determination in biological fluids, and simultaneous kinetic studies etc.

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. 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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