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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SPARFLOXACIN IN BULK AND TABLET DOSAGE FORM BY UV-SPECTROSCOPY

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Abstract:
Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.
Key Words: API, quality control, Research and development (R&D), excipient

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INTRODUCTION:

It should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements.

1. Established identity and purity.
2. Established bio availability/dissolution.

Analytical chemistry

A branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis).The techniques commonly used are titration, precipitation, spectroscopy, chromatography

Analytical chemistry serves the needs of many fields:

- ✓ In industry, analytical chemistry provides the means of testing raw materials and for assuring the quality of finished products whose chemical composition is critical. Many household products, fuels, paints, pharmaceuticals, etc. are analyzed by the procedures developed by analytical chemists before being sold to the consumer.
- ✓ The nutritional value of food is determined by chemical analysis for major components such as protein and carbohydrates and trace components such as vitamins and minerals. Indeed even the calories in a food are often calculated from its chemical analysis.
- ✓ In medicine, analytical chemistry is the basis for clinical laboratory tests, which help physicians to diagnose disease and chart the progress in recovery.
- ✓ Environmental quality is often evaluated by testing for suspected contaminants using the techniques of analytical chemistry.
- ✓ Analytical chemists also make important contributions to fields as diverse as forensic chemistry, archaeology, and space science[1].

Chromatography:

Chromatography is a family of techniques for the seperation. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample.

When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time."

A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.

Chromatograph separates the chemical mixture either liquid or gas into its components by differential distributions of the solutes, as they flow with different rate over the stationary phase. Type of the technique used for the separation of complex mixtures depends on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species[2].

1.1 Detectors

The sensitivity of universal detector for HPLC has not been devised yet. Thus it is necessary to select a detector on the basis of the problem.

UV visible photometers and spectrometers

Optical detectors based on UV -visible absorption are the workhorses of HPLC, constituting over 70% of the all-detection systems in use. Basically, three types of absorbance detectors are available: a fixed wavelength detector, a variable wavelength detector, and a scanning wavelength.

Fixed Wavelength Detectors

A fixed wavelength detector uses a light source that emits maximum light intensity at one or several discrete wavelengths that are isolated by appropriate filters.

Variable Wavelength Detector

This is relatively wide band pass it offers a wide range of selection of UV and visible wavelengths but it is costly one compared to fixed wavelength detectors.

Photo Diode Array (PDA) Detector

Digital electronic integrators are widely used today in HPLC for measuring peak areas. These devices automatically sense peaks and print out the areas in numerical forms. Computing integrators are even more sophisticated and offer a number of features in addition to basic digital integration because these devices have both memory and computing

capabilities to upgrade integrating parameters to maintain accuracy as the separation progress and eluting peaks become broader. Many of these devices print out a complete report including names of the compounds, retention times, peak areas and area correction factors. With the help of peak area and height values, the peak width can be calculated (considering the peak as a triangle) and it can also be used for the calculation of number of theoretical plates[10].

Introduction to UV/Visible Spectrophotometers

UV/Visible spectrophotometry is a mature and established technique, with inbuilt flexibility to detect and measure millions of compounds (analytes) in a wide variety of sample matrices. This technique is used within a wide variety of analytical chemistry laboratories, such as within the following sectors: -

- Life Science commercial enterprises.
- Research and Teaching.
- University, Life sciences, Chemistry.
- Hospitals and Clinics.
- Food and Drinks manufacturing.
- Environmental.
- Water Suppliers.
- Forensics.
- Pathology.
- Pharmaceuticals.
- Nutraceutical

EXPERIMENTAL WORK

METHOD DEVELOPMENT AND VALIDATION BY ULTRAVIOLET **SPECTROPHOTOMETRY**

Requirements

Table 1: Requirements for Analysis of Sparfloxacin by UV spectrophotometry

Requirement	Manufacturer/ Source
Calibrated UV visible spectrophotometer	Shimadzu UV – 1800
Calibrated electronic balance	Mettler Toledo
Sparfloxacin	Cipla Ltd
Volumetric flasks(10, 25, 1000 ml)	Class BBorosil
Pipettes (2, 5, 10 ml)	Class BBorosil
Beakers (50, 100, 250 ml)	Borosil

choice of

- Shimadzu UV-1800 double beam spectrophotometer with matched pair of 10mm quartz cells was used throughout the experimental work.
- UV Vision Pro 2.34 software was used to acquire data. The instrument was used after getting stabilized.
- The instrument parameters viz. start wavelength, end wavelength data, scan speed, slit width and sample information were entered and SPECTRUM method was chosen.
- AUTO ZERO was performed to nullify the absorbance value. BASELINE CORRECTION was done by placing the blank (2mL Methanol & 8mL of 0.1M Sodium hydroxide solution) in both the sample and reference compartments to nullify solvent's effect on absorbance.

Choice of solvent

- With reference to Official compendia (E.P, B.P), it was found that Sparfloxacin is sparingly soluble in water and freely soluble un methanol.
- Solubility check of the drug in various Buffers like 0.1M Hydrochloric acid and 0.1M NaOH
- Solubility check of the drug in various solvents like Methanol and Ethanol
- Revealed the solubility of drug in Methanol owing to the weak basic nature of drug
- Hence Methanol used for the solubility of the drug remaining quantity was diluted with 0.1M Sodium hydroxide was chosen as solvent for UV spectrophotometric analysis which gave distinct spectrum with Gaussian distribution and good absorbance.

Determination of λ_{max}

By trial and error, the λ_{max} of Sparfloxacin in methanol and 0.1N sodium hydroxide by UV spectrophotometer was found to be **295nm.**

Method Validation

Linearity and Range

Linearity for the concentration range 4- 20µg/ ml was established by plotting concentrations on X- axis and corresponding absorbance on Y- axis. Statistical parameters like correlation coefficient (R^2), line equation including slope (m), y- intercept (C) were determined.

The specified range was derived from linearity studies by determining the difference between

highest and lowest concentrations.

Precision

Intraday precision (Repeatability)-Repeatability of the developed method was assessed by 9 determinations covering 3 concentrations each of 3 replicates. % RS was calculated for the results obtained.

Inter day Precision-Variations in the results for the developed method was assessed amidst 3 different days (n= 6). % RSD was calculated for the results obtained.

Limit of Detection and Limit of Quantitation

LOD and LOQ were determined by instrumental methods based on the standard deviation of the response (blank sample) and slope of the calibration curve.

Accuracy

Accuracy of the method was confirmed by recovery studies from marketed formulation at three levels of standard addition from 50%, 100% and 150 % of label claim. The recovery studies were carried out in triplicate.

Preparation of 50% solution:0.5ml of sample stock solution (5µg/ ml) and 0.25 ml of above standard stock solution II were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 100% solution: 0.5ml of sample stock solution (5µg/ ml) and 0.5 ml of above standard stock solution II were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 150% solution:0.5 ml of sample stock solution (5µg/ ml) and0.75ml of above standard stock solution II were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Applicability of the Developed Validated Method by Ultraviolet Spectrophotometry

Assay of Formulation

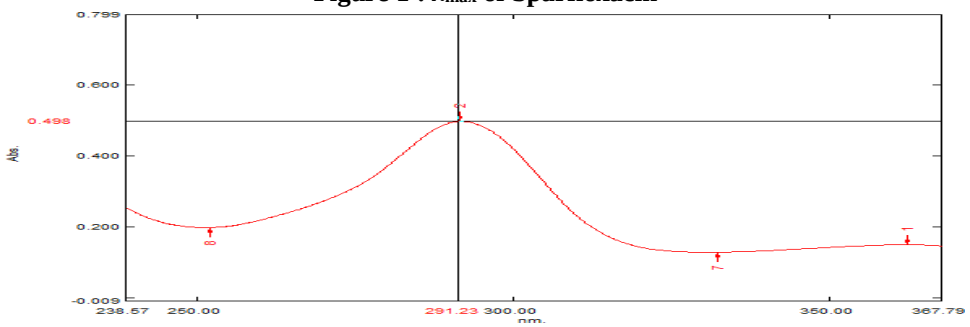
- 20 tablets were weighed accurately and average weight of tablet was noted that constitutes 75 mg Sparfloxacin and was finely powdered.
- The tablet powder equivalent to 100mg of Sparfloxacin was accurately weighed and transferred to 10 ml volumetric flask and dissolved in about 3 ml of the solvent (Methanol).
- It was then vortexed for 30 minutes to enhance maximum extraction of the active pharmaceutical ingredient from the dosage form and filtered through Whatmann No 1 filter paper to remove insoluble excipients to the maximum extent.
- It was then made up to the volume with the 0.1M Sodium hydroxide solution. This constitutes 12µg/ ml of Sparfloxacin.
- From the stock solution, aliquot corresponding to medium concentration of standard curve was prepared and made upto the mark with the solvent.
- The absorbance was noted, and the corresponding concentration was then determined from the standard calibration curve.

RESULTS AND DISCUSSION:

METHOD DEVELOPMENT AND VALIDATION BY ULTRAVIOLET SPECTROPHOTOMETR

Determination of λ_{max} of Sparfloxacin

Figure 1 : λ_{max} of Sparfloxacin



Method Validation

Linearity

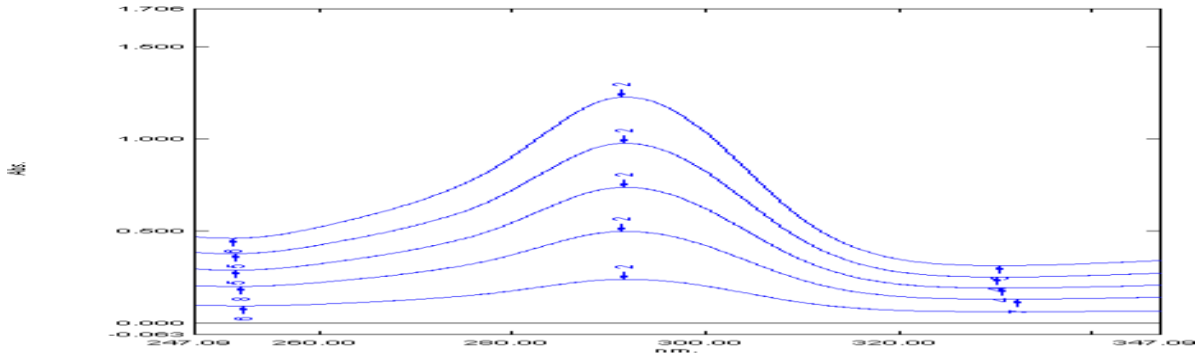


Figure 2: Overlay Spectra of Sparfloxacin (4-20 µg/ ml)

Table 2: linearity Profile by UV Spectrophotometry

Concentration (µg/ ml)	Absorbance at 291.00 nm
4	0.237
8	0.498
12	0.736
16	0.976
20	1.226

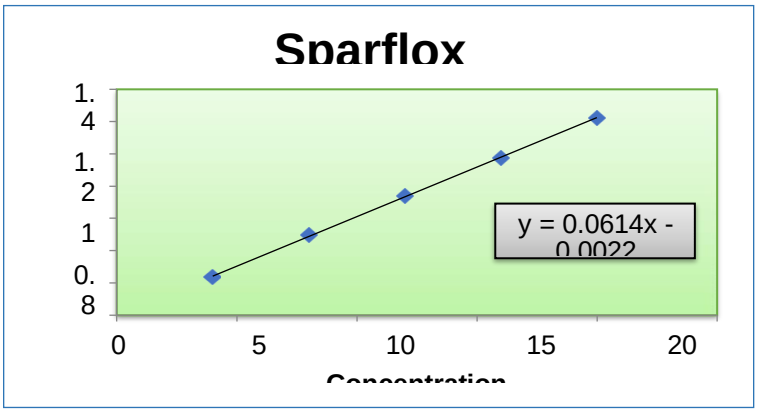


Figure 3: standard Calibration Curve for the Linearity Set at 291.00 nm by UV Spectrophotometry

Table 3: summary of Regression Equation by UV spectrophotometry

Line equation	y= 0.0614x-0.0022
correlation coefficient (R^2)	0.9998
y- intercept (C)	0.0022
Slope (m)	0.0614

The results obtained were within the range from the linearity set.

Precision

Intra-day precision (Repeatability)

Table 4: Intra-day Precision Day- I by UV Spectrophotometry

Conc (µg/ ml)	Absorbance			Average	SD[a]	% RSD[b]
	Set 1	Set 2	Set 3			
4	0.236	0.238	0.235	0.236333	0.001528	0.646344
8	0.496	0.499	0.497	0.497333	0.001528	0.307143
12	0.735	0.737	0.736	0.736	0.001	0.13587

[a]= Standard Deviation, [b]= Percentage Relative Standard Deviation

Table Intra-day Precision Day- II by UV Spectrophotometry

Conc (µg/ ml)	Absorbance			Average	SD	% RSD
	Set 1	Set 2	Set 3			
4	0.237	0.236	0.235	0.236	0.001	0.423729
8	0.497	0.498	0.496	0.497	0.001	0.201207
12	0.737	0.735	0.736	0.736	0.001	0.13587

Table 5: Intra-day Precision Day- III by UV Spectrophotometry

Conc (µg/ ml)	Absorbance			Average	SD	% RSD
	Set 1	Set 2	Set 3			
4	0.236	0.235	0.238	0.2363	0.001528	0.646344
8	0.497	0.498	0.499	0.498	0.001	0.200803
12	0.735	0.736	0.737	0.736	0.001	0.13587

Accuracy

Figure Overlay Accuracy Spectra of SPARFLOXACIN by UV Spectrophotometry

Recovery from Formulation (Sparfloxacin tablets) by UV spectrophotometry

Sparfloxacinin dosage form (µg ml^{-1})	% Pure Sparfloxacin added	Pure Sparfloxacin Added (µg ml^{-1})	Sparfloxacin Recovered% ± %RSD*
5	50%	2.5	101.02± 1.17
5	100%	5	101.90± 1.12
5	150%	7.5	102.00± 0.17

*Acceptance Criteria:*The % Recovery for each level should be between 98.0 and 102.0%

- The developed method was found to be accurate since % recovery for each level was within limits and RSD less than 2.0.

Applicability of the Developed Validated Method by Ultraviolet Spectrophotometry

Assay of formulation

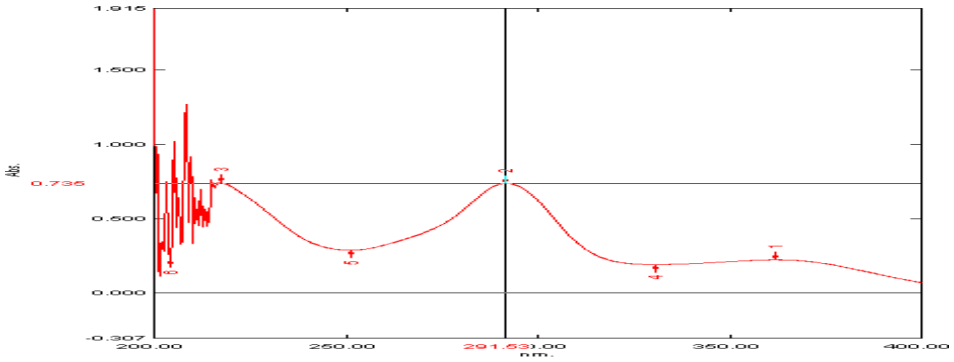


Figure4: Overlay Assay Spectra of Sparfloxacin by UV Spectrophotometry

Table 6: Assay of formulation (Sparfloxacin 200mg tablets) by UV spectrophotometry

Acceptance criteria: 95- 105% w/v The assay results were decorous in conjunction with acceptance criteria

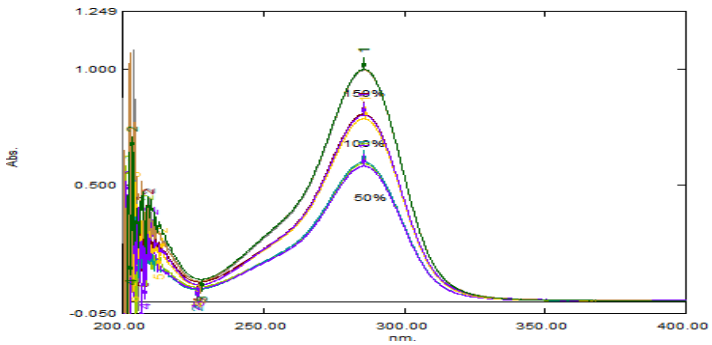
Formulation	Absorbance	Label claim	Amount found	% Assay ± SD*
Sparfloxacin	0.741	200 mg	11.934	99.45%
	0.735			
	0.731			

Inter-day precision

Table 7: Inter-day Precision by UV Spectrophotometry

Conc (µg/ ml)	Absorbance						Average	SD	% RSD
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6			
4	0.238	0.235	0.236	0.237	0.235	0.236	0.23616	0.001169	0.495009
8	0.499	0.497	0.498	0.497	0.498	0.496	0.4975	0.001049	0.210816
12	0.737	0.736	0.735	0.737	0.736	0.735	0.736	0.000894	0.121525

✓ The developed method was found to be precise as the % RSD of the results within and amidst 3 days was within limits (< 2.0).



Accuracy

Figure Overlay Accuracy Spectra of SPARFLOXACIN by UV Spectrophotometry
Recovery from Formulation (Sparfloxacin tablets) by UV spectrophotometry

Sparfloxacinin dosage form (µg ml^{-1})	% Pure Sparfloxacinadded	Pure Sparfloxacin Added (µg ml^{-1})	Sparfloxacin Recovered% ± %RSD*
5	50%	2.5	101.02± 1.17
5	100%	5	101.90± 1.12
5	150%	7.5	102.00± 0.17

*Acceptance Criteria:*The % Recovery for each level should be between 98.0 and 102.0%

The developed method was found to be accurate since % recovery for each level was within limits and RSD less than 2.0 Applicability of the Developed Validated Method by Ultraviolet Spectrophotometry

Assay of formulation

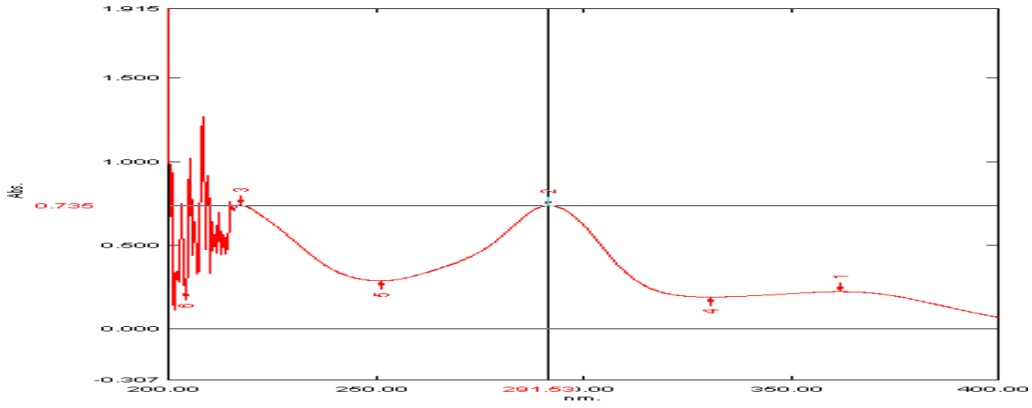


Figure 5: Overlay Assay Spectra of Sparfloxacin by UV Spectrophotometry

Table 8: Assay of formulation (Sparfloxacin 200mg tablets) by UV spectrophotometry

Acceptance criteria: 95- 105% w/v The assay results were decorous in conjunction with acceptance criteria

Formulation	Absorbance	Label claim	Amount found	% Assay ± SD*
Sparfloxacin	0.741	200 mg	11.934	99.45%
	0.735			
	0.731			

DISCUSSION:

A simple and selective UV method is described for the determination of Sparfloxacin. Linearity was observed in the range 4-20µg /ml for Sparfloxacin (r^2 =0.999) for the amount of drug estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form

CONCLUSION

From the above experimental results and parameters

it was concluded that, this newly developed method for the simultaneous estimation of Sparfloxacin was found to be simple, precise, accurate and high resolution makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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