Development and Validation of a Stability-Indicating RP-UPLC Method for the Quantitative Analysis of Sparfloxacin

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Abstract

A rapid, specific, and sensitive ultra-performance liquid chromatographic (UPLC) method for quantitative analysis of sparfloxacin in bulk drug and pharmaceutical formulations has been developed and validated. In this work, a new gradient reversed-phase chromatographic method was developed. The newly developed method is applicable for assay determination of the active pharmaceutical ingredient. The chromatographic separation of sparfloxacin was achieved on a Waters Acquity HSS T-3 column (100 × 2.1 mm, 1.8 µm) within a short runtime of 5 min. The method was validated according to the ICH guidelines with respect to system suitability, linearity, limit of quantitation and detection, precision, accuracy, robustness, and specificity. Forced degradation studies were also performed for sparfloxacin bulk drug samples to demonstrate the stability indicating power of the UPLC method. Comparison of system performance with conventional HPLC was made with respect to analysis time, efficiency, and sensitivity. The developed method was applied for the assay of marketed sparfloxacin formulations like tablets and eye drops.

Introduction

High-performance liquid chromatography (HPLC) is a proven technique that has been used in laboratories worldwide for at least 30 years. According to the Van Deemter equation using smaller particles, the speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed ultra-performance liquid chromatography (UPLC). Though HPLC is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (APIs) and dosage forms, it is often a slow technique because of the complexity of some of the samples. At a time when many scientists have reached separation barriers with conventional HPLC, UPLC presents the possibility to extend and expand the utility of liquid chromatography. UPLC, which utilizes sub-2 µm particles for stationary phase at a maximum operating pressure of 15,000 psi (compared with conventional HPLC on 5 µm particles at 5000 psi), has proved to be a suitable analytical technique which fulfills the promise of increased resolution, speed, and sensitivity predicted for liquid chromatography (1,2). Because of its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceutical and biomedical analysis (3–6). In the present work, this technology has been applied to the method development and validation study of assay determination of sparfloxacin bulk drug.

Sparfloxacin, cis-5-amino-l-cyclopropyl-7,(3-5-dimethyl-1-piperazinyl)-6-8-difluoro-1-4-dihydro-4-oxo-3-quinoline carboxylic acid (Figure 1), is a novel antibacterial drug belonging to the third generation fluroquinoline group of drugs. Sparfloxacin is reported to be more active in vitro than ciprofloxacin against mycobacteria and Gram-positive bacteria, including Streptococcus pneumoniae and other streptococci and staphylococci (7). It is an INN drug, and as such it has not been included in the USP and B.P. A number of analytical methods (8–15) have been developed for the analysis of sparfloxacin for research purposes. Of these, the most widely used method for the analysis of sparfloxacin is based on HPLC.

In this work, we show how the HPLC method for sparfloxacin has been transferred to UPLC. A comparison was made between HPLC and UPLC efficiency on the basis of resolution and sensitivity. The developed stability indicating reversed-phase (RP)-UPLC method was then validated as per ICH guidelines along with application of the developed method in the assay of marketed sparfloxacin formulations like tablets and eye drops.

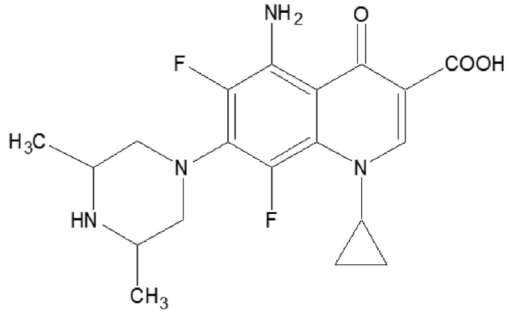

Figure 1. Structure of sparfloxacin.

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Experimental

Materials and reagents

The drug sparfloxacin (99.25% pure on dried basis) was received as a gift sample from Micro Labs (Bangladore, India). HPLC-grade acetonitrile was purchased from Qualigens Fine Chemical (Mumbai, India). Orthophosphoric acid (OPA) was purchased from Merck India Ltd. (Mumbai, India). High-purity water was obtained by Millipore Milli-Q water purification system (Billerica, MA). All other reagents are of analytical-grade.

High-performance liquid chromatography

The HPLC system used for initial chromatographic development was a Waters Alliance separation module with a 2487 UV detector (Milford, MA). A Kromasil C_{18} column (250 × 4.6 mm, 5 µm) was used for separation. Mobile phase consisting of a mixture of A: 0.1% aqueous OPA and B: acetonitrile (ACN) is used. The timed gradient program: T (min)/%B: 0/10, 7/10, 13/25, 19/25, 25/10, and 31/10 with the flow rate of 1 mL/min was employed. The injection volume was 12 µL while detector was set at 290 nm. The column was maintained at 50ºC.

Ultra-performance liquid chromatography

UPLC was performed using a Waters Acquity system equipped with binary solvent delivery pump, an autosampler, and tunable UV detector. The chromatographic separation was performed using a Waters Acquity HSS T-3 C_{18} column (100 × 2.1 mm, 1.8 µm). The mobile phase containing a mixture of 0.1% aqueous OPA on a timed gradient program: T (min)/%B: 1/10, 2/10, 3/25, 4/10, and 5/10 with the flow rate of 0.5 mL/min was used. The detection was obtained at a wavelength of 290 nm. The injection volume was 1 µL. ACN solution was used as a diluent while the column was maintained at 50°C. Forced degradation studies were carried out with a Waters 2996 photodiode array detector.

Preparation of solution

A stock solution of 1.0 mg/mL was prepared by dissolving appropriate amount of sparfloxacin in diluent. Working solution of 100 µg/mL was prepared from stock solution for assay determination.

System suitability

Solution was prepared by dissolving 10 mg of sparfloxacin in 100 mL of ACN. The test solution (100 µg/mL) was prepared by dissolving the appropriate amount of sparfloxacin in diluent. This standard solution was also used as system suitability solution. For the calibration of the assay method, different concentration of stock solutions ranging from 80–120 µg/mL were analyzed, and calibration curve was plotted against the peak areas and analyte concentrations.

Table I. Differences in Performances of HPLC and UPLC

System	Elution time (min)	Flow rate (mL/min)	Injection volume (µL)	Run time (min)	Tailing factor	USP plate count
HPLC	20.2	1	12	31	1.5	14,263
UPLC	2.746	0.5	1	5	1.17	41,250

Validation procedure

The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. The newly developed UPLC method was validated according to International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (16–19). The method was validated with respect to linearity, limit of detection (LOD) and limit of quantification (LOQ), precision, accuracy, robustness, and specificity (20).

The LOD and LOQ for API were estimated by injecting a series of dilute solutions with known concentration. The LOQ was estimated from visual evaluation and signal-to-noise ratio. Assay method precision was carried out using six independent test solutions and a standard preparation. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was eval-

Table II. Results of Regression Analysis of Linearity Data of Sparfloxacin Bulk Drug

Parameters	Results
Range (µg/mL)	80–120
Slope	0.67996.6
Intercept	–4935873616
R^2	0.999
LOD (µg/mL)	0.2 µg/mL
LOQ (µg/mL)	0.6 µg/mL

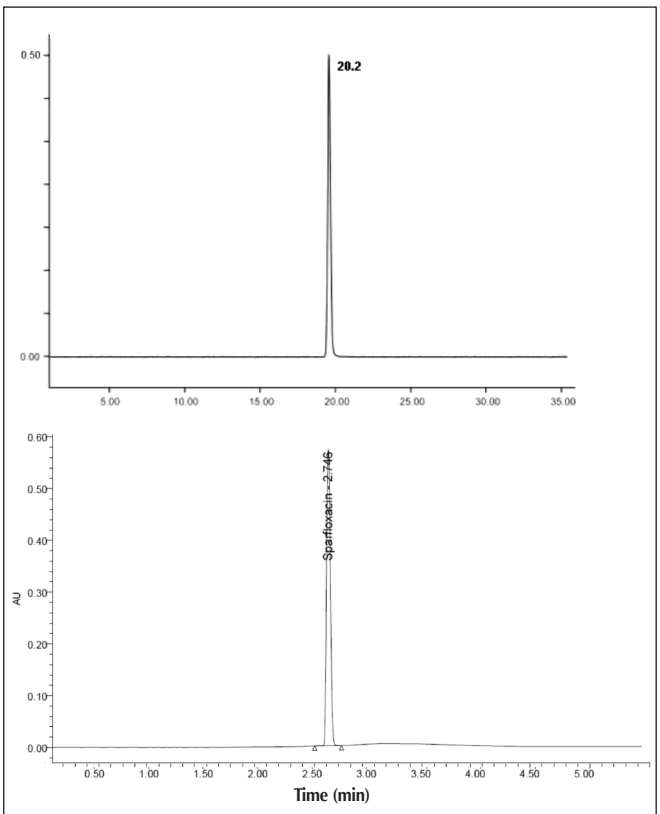

Figure 2. Comparison of chromatograms (A) HPLC and (B) UPLC of sparfloxacin.

uated in triplicate using three concentration levels: 80, 100, and 120 µg/mL. Recovery experiments were conducted to determine accuracy of the method. To determine the robustness of the method, experimental conditions were purposely altered and examined by injecting system suitability solution. The flow rate was changed to 0.45 and 0.55 mL/min. Column temperature was varied by ± 2°C (i.e., 48°C and 52°C). The wavelength number was varied by ± 2 nm.

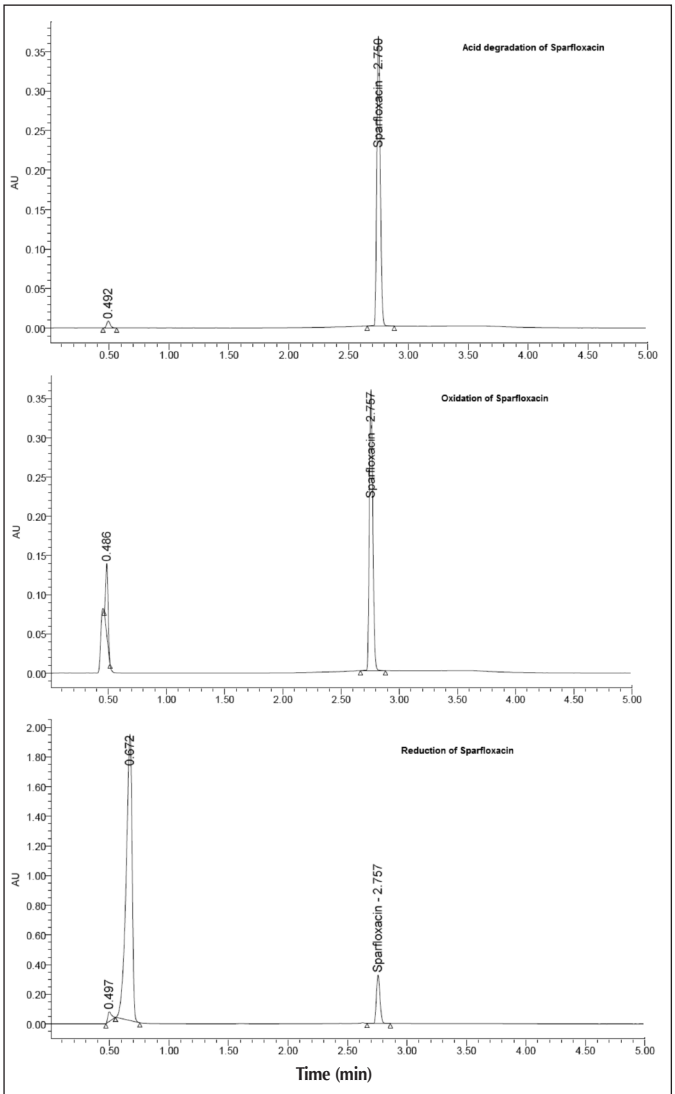

Figure 3. UPLC chromatograms of sparfloxacin stressed samples: (A) acid degeneration, (B) oxidative degeneration, and (C) reductive degeneration.

Table III. Intermediate Precision and Repeatability Data

	Intermediate precision				Repeatability	
	Inter-day measured conc.		Different analyst measured conc.		Intra-day measured conc.	
Levels (µg/mL)	Avg. conc. rec.* (µg/mL)	% RSD	Avg. conc. rec. (µg/mL)	% RSD	Avg. conc. rec. (µg/mL)	% RSD
80	79.82	0.06	79.69	0.08	79.78	0.08
100	100.29	0.01	99.92	0.06	100.28	0.05
120	119.80	0.05	119.89	0.03	119.90	0.09

* Average concentration recovered.

Forced degradation studies of the bulk drug sample were also performed using the following conditions: acid hydrolysis (0.1 N hydrochloric acid), base hydrolysis (0.1 N sodium hydroxide), heat (105°C for 96 h), photolytic (UV and sunlight for 96 h), oxidation (30% hydrogen peroxide), and reduction (10% sodium metabisulphite). Peak purity test was carried out for sparfloxacin peak by using a photodiode array detector in stress samples.

Estimation from formulations

Tablets

Ten tablets were weighed and pulverized. Amount of the powder equivalent to 100 mg of sparfloxacin was taken and extracted with diluent separately for 30 min. This solution was diluted suitably to prepare a 100 µg/mL concentration. Finally, solutions were filtered through Whatman filter paper number 40, and the filtrate was suitably diluted with diluent to prepare a 100 µg/mL concentration. The samples were analyzed using proposed analytical method.

Eye drops

Three mL of sparfloxacin eye drop solution equivalent to 9 mg of sparfloxacin was taken and suitably diluted with diluent separately to get a 90 µg/mL concentration. The samples were analyzed using proposed analytical method.

Results and Discussions

LC method development and transfer to UPLC

The main target of the chromatographic method was to achieve separation and quantification of sparfloxacin. Initially, the gradient HPLC conditions were optimized for sparfloxacin in bulk drug, which was then transferred to UPLC. Gradient system is always preferred over isocratic system in order to achieve improved peak shape and resolution. With the isocratic system, sometimes peak is eluted late so the gradient system was used to reduce run time. Hence, it was decided to use gradient HPLC mode. The response of sparfloxacin was found to be adequate at 290 nm. The HPLC chromatographic separation was achieved on a Kromasil C_{18} column (250 × 4.6 mm, 5 µm) maintained at 50ºC. The basic chromatographic conditions such as stationary phase, solvents, and UV detection employed in HPLC were taken into account while developing the new UPLC method. The detection wavelength, column temperature, and buffer and solvent used in HPLC were kept constant. The stationary phase C_{18} was chosen in order to have similar chemistry as that used in the HPLC. Primary a BEH C_{18} column (100 × 2.1 mm, 1.7 µm) was used, but the peak found was distorted and not acceptable. The BEH C_{18} column was then replaced with a HSS T-3 column (100 × 2.1 mm, 1.8 µm). The peak found was sharp and acceptable. The injection volume in UPLC was scaled to 1 µL from 12 µL as used in HPLC whereas the mobile phase containing a mixture of 0.1% aqueous OPA remained the same on a timed gradient program T (min)/%B: 1/10, 2/10, 3/25, 4/10, and 5/10 with the flow rate of 1 mL/min was used.

Using these conditions, a satisfactory peak was achieved for sparfloxacin eluting around 2.74 min, giving a total run time of 5 min.

Comparison study of chromatographic performance

A comparative data on chromatographic performance of HPLC and UPLC has been obtained by injecting a solution of sparfloxacin (100 µg/mL). The performance parameters of both the systems are shown in Table I. It is observed that the elution time of sparfloxacin in UPLC was reduced 10-fold to that of gradient mode HPLC. The resolution and theoretical plates obtained for sparfloxacin in UPLC showed comparatively better separation efficiency than HPLC. Theoretical plates obtained for UPLC is three-fold higher then for HPLC with reduced tailing of 1.17. The typical chromatograms obtained from final HPLC and UPLC conditions are depicted in Figure 2.

UPLC method validation

The aim of the validation study was to confirm the method suitability for its intended purpose of routine analysis. The assay values of different bulk drug samples were found to be in the range of 80–120%. Forced degradation studies were also performed for sparfloxacin bulk drug sample to demonstrate the stability-indicating power of the newly developed UPLC method.

System suitability

System suitability test were used to verify that the proposed method was able to produce good resolution between the peaks of interest with high reproducibility. System suitability is analyzed in terms of tailing factor (must be < 1.5), theoretical plate counts (should be > 20000), retention time, etc. The result for proposed UPLC method is given in Table I. According to the results presented, the proposed UPLC method fulfils these requirements within the accepted limits.

Linearity

A linear calibration plot for the sparfloxacin was obtained at five concentration levels (80–120 µg/mL) in triplicate. Regression equation is $y = 6996.6x - 73616$ with the correlation coefficient (R^2) greater than 0.999. The results showed excellent correlation between the peak area and concentration of sparfloxacin. Results obtained from regression analysis of the linearity data for sparfloxacin bulk drug is summarized in Table II.

Table IV. Recovery Analysis of Sparfloxacin

Conc.	Actual (µg/mL)	Recovered (µg/mL)	Recovery (%)	RSD (%)
80% level	80.52	80.41	99.9	0.45
of test conc.	80.34	79.89	99.4	
	80.43	80.69	100.3	
100% level	103.28	103.86	99.4	0.74
of test conc.	104.27	105.23	99.1	
	103.75	103.21	100.5	
120% level	123.48	124.12	99.5	0.38
of test conc.	124.63	125.32	99.4	
	124.00	125.56	98.8	

LOQ and LOD

LOQ was determined at a sound-to-noise ratio of ≥ 10 whereas LOD was determined at sound-to-noise ratio of ≥ 3. LOQ value for sparfloxacin was found to be 0.6 µg/mL with a sound-to-noise ratio of 12–15. The LOD values for sparfloxacin bulk drug was found to be 0.2 µg/mL with a sound-to-noise ratio of 7. Data is represented in Table II.

Precision

The precision of the assay method was evaluated as repeatability and intermediate precision by carrying out six independent assays at 80, 100, and 120 µg/mL concentration of sparfloxacin on different days by different analysts. For repeatability, the % relative standard deviation (% RSD) of sparfloxacin was found to be in a range of 0.05–0.09 Table III) whereas the % RSD of assay results obtained in intermediate precision study was in the range

Table V. Robustness Evaluation of the Developed UPLC Method

Chromatographic changes	Sparfloxacin* %RSD (Peak Area) $n = 6$	Tailing factor	Theoretical plate count
Flow rate (mL/min)			
0.45	0.5%	1.18	40288
0.5†	0.5%	1.17	41250
0.55	0.4%	1.15	42580
Temperature (°C)			
48	0.5%	1.17	40728
50†	0.5%	1.17	41250
52	0.4%	1.16	42329
UV Wavelength (nm)			
288	0.4%	1.16	42793
290†	0.5%	1.17	41250
292	0.3%	1.16	42815

* All values given are taken as mean of 6 individual values. †Optimum condition.

Table VI. Summary of Forced Degradation Studies

Stress condition	Time	Purity angle	Purity threshold	Remark
Acid hydrolysis (1 N HCl at RT)	8 h	0.095	0.284	Some degradation was observed, but main peak is homogeneous.
Base hydrolysis (1 N NaOH at RT)	8 h	0.128	0.294	No degradation. Main peak is homogeneous.
Oxidation (30% H_2O_2 at RT)	8 h	0.097	0.276	Degradation was observed, but the main peak is homogenous.
Reduction (10% $Na_2S_2O_5$)	8 h	0.559	0.855	Degradation was observed, but the main peak is homogenous. Degraded product was eluted separately.
Thermal (105°C)	24 h	0.069	0.292	No degradation was found.
	48 h	0.054	0.286	
	72 h	0.065	0.290	
	96 h	0.064	0.287	
Sunlight	24 h	0.085	0.283	No degradation was found.
	48 h	0.095	0.290	
	72 h	0.099	0.291	
	96 h	0.090	0.287	

of 0.01–0.08 (Table III). These % RSD values are well within the generally acceptable limit of 2%, confirming good precision of the assay method.

Accuracy

The accuracy of the method was determined for sparfloxacin by recovery experiments. Known amount of sparfloxacin bulk sample (test preparation) in triplicate at levels 80%, 100%, and 120% of the specified limit were taken for analysis. Results obtained from recovery studies are given in Table IV. The accuracy of the assay method was evaluated in triplicate at three concentration levels, 80, 100, and 120 µg/mL, in bulk drug sample. The percentage recovery of sparfloxacin in bulk drug samples ranged from 99.1 to 100.5% with % RSD of values of 0.45, 0.74, and 0.38% at 80, 100, and 120% level, respectively (Table IV). High recovery results obtained from the proposed UPLC assay method indicates that this method can be used for quantitative routine quality control analysis of pharmaceutical dosage form.

Robustness

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in analytical method (e.g., flow rate, column temperature, and UV detector wavelength). Changes in chromatographic parameters (i.e., theoretical plates and tailing factor) were evaluated for the studies. In all the deliberately varied chromatographic conditions, the chromatogram for system suitability solution showed satisfactory resolution (RSD < 2%) with no significant changes in chromatographic parameters (Table V).

Specificity

Stress studies of the drug APIs is utilized for identification of the possible degradation products and for the validation of the stability-indicating analytical procedures. It is the ability of analytical method to measure the analyte response in the presence of its degradents. The result obtained from the forced degradation studies is summarized in Table VI. During the forced degradation study, a considerable degradation of drug substance was observed in acidic, oxidative, and reductive conditions (Figure 3). The chromatograms were checked for the appearance of any extra peaks. Peak purity of these samples under stressed conditions was verified using a photodiode array detector (Figure 4). The purity of the principle and other chromatographic peaks was found to be satisfactory. This study confirmed the stability-indicating power of the UPLC method.

Estimation of formulations

The applicability of the validated method was also tested by analyzing sparfloxacin in pharmaceutical dosage forms (e.g., tablets and eye drops). The assay values of sparfloxacin for different formulations represent the average of six individual assays. Sparfloxacin tablet, which was claimed to contain 200 mg of sparfloxacin, gave a mean assay value of 199.2 ± 1.1097 (standard deviation, SD) mg. Recovery and % RSD of the assay method were 99.6% and 0.56% respectively. On the other hand, sparfloxacin eye drop, which was claimed to contain 0.3% w/v of sparfloxacin, gave a mean assay value of 3.2 ± 0.0987 mg/mL with a recovery and % RSD of 107.8 and 3.05, respectively. Assay values of formulations were found near by claimed value, indicating that the interference of excipient matrix is insignificant in estimation of sparfloxacin by proposed UPLC method. The estimated drug content with low values of RSD (< 1%) established the precision of the proposed methods. The results obtained from the analytical method are compiled in Table VII.

Table VII. Application of UPLC Method to the Determination of Sparfloxacin from Pharmaceutical Dosage Forms

Commercial formulations	Labelled claim (mg)	Mean amount found	No. of samples	SD of the amount found	RSD % of the amount found	Recovery %
Sparx 200 tablets	200 mg	199.2 mg/tablet	6	1.1097	0.56	99.6%
SparDrops SCAT Eye drop (0.3% w/v)	3 mg/mL	3.2 mg/mL	6	0.0987	3.05	107.8%

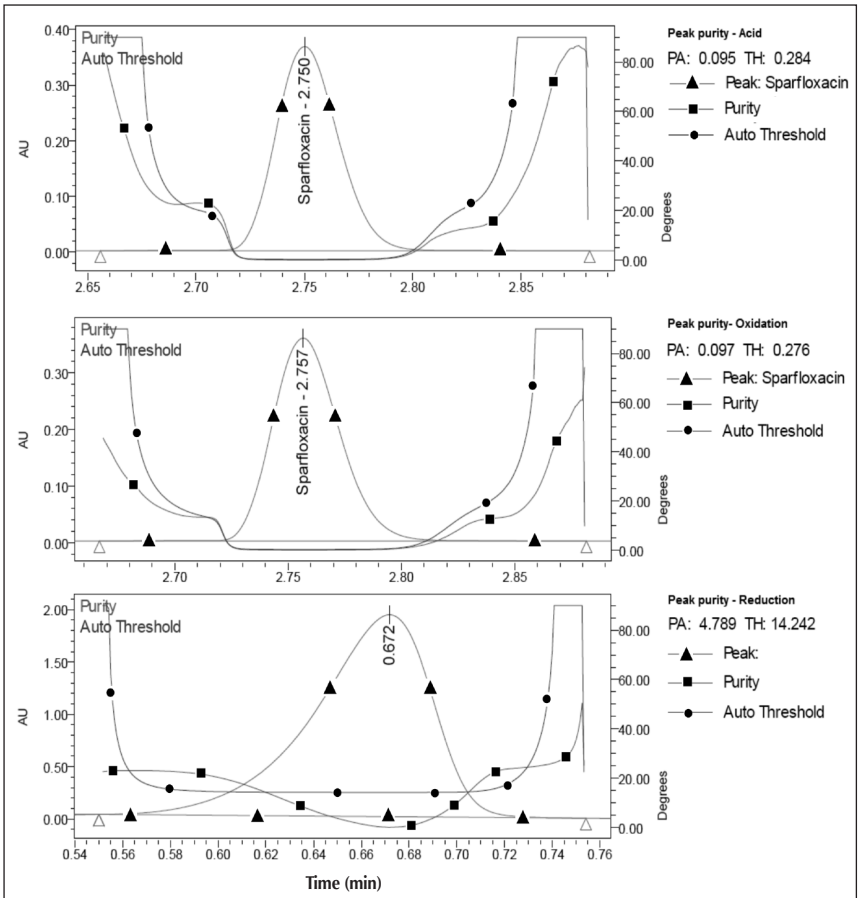

Figure 4. Photodiode array detection peak purity: (A) acid degeneration, (B) oxidative degeneration, and (C) reductive degeneration.

Conclusion

The newly developed stability indicating RP-UPLC method for assay determination of sparfloxacin was found to be capable of giving faster retention times maintaining good resolution than that achieved with conventional HPLC. The method was completely validated showing satisfactory data for all the parameters tested. This method exhibited an excellent performance in terms of sensitivity and speed. It is a stability-indicating method suitable for rapid analysis of sparfloxacin bulk drug and its pharmaceutical formulations.

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