STABILITY-INDICATING HPLC AND UV SPECTROPHOTOMETRIC DETERMINATION OF SOFOSBUVIR IN PURE FORM AND TABLETS

BY

Ahmad Abdelhalim Abouserie Mohamad*

FROM

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, 11751, Nasr City, Cairo, Egypt

ABSTRACT

Four simple, sensitive, accurate and precise stability-indicating methods were developed for determination of sofosbuvir (SFB) in pure form as well as in its pharmaceutical preparation and in presence of its alkaline degradate. The first method is an HPLC stability–indicating method, where the intact drug (SFB), the internal standard (atorvastatin) and SFB degradation product were separated on a Athena C_{18} (250 mm X 4.6 mm ID, 5µm particle size) column using methanol–water (70:30, v/v) as a mobile phase at a flow rate of 1 ml/min and UV detection at 260 nm. The second method is the ratio difference method, where the UV absorption spectra of different concentrations of SFB were divided by the absorption spectrum of a certain concentration (30 µg /ml) of its degradation product (divisor) to get the ratio difference spectra. Afterwards, the difference in peak amplitudes between 270 and 245 nm were measured. The third method is the ratio derivative method, where the amplitudes of first derivative of the obtained ratio difference spectra were measured at 282 nm. The fourth method is the mean centering of ratio difference spectra, where the amplitudes of the mean centered ratio difference spectra were measured at 262.6 nm. The calibration curves were linear over the concentration range of 5-35 µg/ml for all methods. The proposed methods can selectively analyse the drug in presence of up to 86 % of its degradation product with mean recoveries of 100.66±1.310, 101.04±1.662, 101.06±1.026 and 99.92±1.374 for the four methods, respectively. These methods were validated and successfully applied for the determination of SFB in its commercial preparation. Moreover, the obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level. It was found that no significant differences were observed regarding accuracy and precision.

KEY WORDS

Sofosbuvir, Stability-indicating, Ratio difference, Ratio derivative, Mean centering ratio spectra.

INTRODUCTION

SFB (Fig. 1) is (*S*)-Isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4 dihydropyrim-idin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl) (methoxy) - (phenoxy)- phosphorylamino)propanoate. It is a white crystalline solid with a solubility of ≥ 2 mg/mL in pH range of 2-7.7 at 37 °C, freely soluble in methanol and

slightly soluble in water. It is a nucleotide analog inhibitor of hepatitis C virus NS5B polymerase. It is indicated for the treatment of chronic hepatitis C infection as a component of a combination antiviral treatment regimen **(RxList, 2015)**

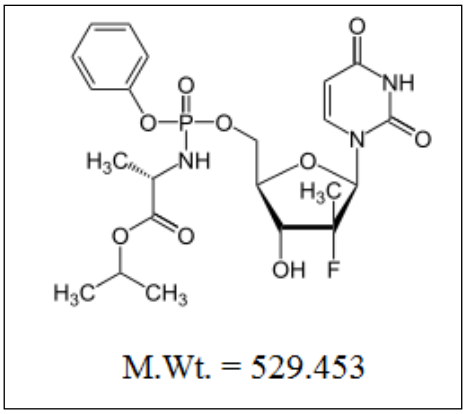

Figure 1: Structural formula of SFB

To our knowledge there is no analytical methods have been published for the analysis of SFB neither in its pharmaceutical preparation nor in presence of its alkaline degradation product.

HPLC is an important qualitative and quantitative technique, generally used to separate, identify, and quantify the active compounds in pharmaceutical and biological samples **(Martin and Guiochon, 2005)**. Reversed-phase chromatography is the most commonly used separation mode in HPLC. The reasons for this include the simplicity, versatility and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass **(Willard and Dean, 1986; Harvey, 2000; Connors, 2005)**.

Under computer-controlled instrumentation, first derivative of ratio spectra, ratio difference and mean centering of the ratio spectra methods are playing a very important role in the analysis of binary mixtures without previous separation by UV–VIS spectrophotometry **(El-Ragehy et al., 2002; Issa et al., 2011; Abdallah and Badawey, 2011; EL-Bagary et al., 2011; Elzanfaly et al., 2012; Lotfy and Hagazy, 2012; Darwish et al., 2013; Lotfy et al., 2012; Belal et al., 2013; Darwish et al., 2011; Afkhami and Bahram, 2004; Afkhami and Bahram, 2005; Afkhami and Bahram, 2006)**

In this work; HPLC, UV ratio difference, UV ratio derivative and UV ratio mean centering methods were applied to the selective determination of SFB in presence of its alkaline degradate. The proposed procedures were successfully applied for determination of SFB in bulk powder and in its pharmaceutical dosage form.

MATERIALS AND METHODS

Apparatus

- HPLC, LDC Analytical (Milton Roy, USA), equipped with Diode-array UV-Visible detector and auto sampler injector. The chromatographic analysis was carried out using (EZ Chrom Elit) data analysis program.
- Shimadzu UV-Vis. 1800 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells.
- Hot plate, Torrey pines scientific (USA).
- Jenway pH meter, 3510 (USA).
- UV lamp with short wavelength 254nm (Vilber Lourmat, France)
- Precoated TLC plates silica gel 60 GF_{254} (20 x20 cm), 0.22mm thickness (Fluka, Chemie, Switzerland).

Pure sample

- SFB was kindly supplied by Al Andalous for Pharmaceutical Industries, 6th of October, Giza, Egypt. The purity was assigned as 99.15%.
- Atorvastatin was kindly supplied by Amoun Pharmaceutical Company, Cairo, Egypt. The purity was assigned as 99.25%.

Pharmaceutical preparation

SOVALDI® tablets, each tablet contains 400 mg of SFB (B.No. 100924, manufactured by Gilead Sciences, USA), purchased from local market.

Reagents and solvents

All chemicals and reagents used throughout the work were of analytical grade.

- Water used throughout the procedures was freshly double distilled.
- Methanol, HPLC grade (Sigma–Aldrich, USA).
- Acetonitrile, HPLC grade (Sigma–Aldrich, USA).
- Chloroform, HPLC grade (Sigma–Aldrich, USA).
- Glacial acetic acid (Fisher Scientific, USA).
- Sodium hydroxide (El-Nasr Company, Egypt), prepared as 1 M aqueous solution.
- Hydrochloric acid (El-Nasr Company, Egypt), prepared as 1 M aqueous solution.
- Potassium dihydrogen orthophosphate (El-Nasr Company, Egypt)
- Orthophosphoric acid 85% (peypin, France).
- Phosphate buffer pH 3.5 **(British Pharmacopoeia, 2012).**

Standard solutions:

Standard solution of intact SFB

A standard solution of SBF (100 μg/ml) was prepared by dissolving 10 mg of SFB in 50 ml of methanol and complete to 100 ml with the same solvent.

Standard solution of atorvastatin (Internal standard):

A standard solution of atorvastatin (100 μg/ml) was prepared by dissolving 10 mg of atorvastatin in 50 ml of methanol and complete to 100 ml with the same solvent.

Standard solution of degraded sample:

100 mg of pure SFB powder were refluxed with 50 ml 1M NaOH for 38 hours. After cooling, the solution was neutralized by 1M HCl, evaporated to dryness under vacuum. The obtained residue was extracted with methanol (2 × 10 ml), filtered into a 100-mL volumetric flask and diluted with methanol to obtain a stock solution labeled to contain degradate derived from 1 mg/ml of SFB. On the other hand, SFB showed no considerable degradation when acidic, oxidative and UV degradation methods had been used instead of alkaline.

Procedures

Construction of the calibration curve (General procedure)

HPLC method

Chromatographic conditions

At ambient temperature, isocratic separation was carried out on Athena C18 (250 X 4.6 X 5µm particle size) using mobile phase consists of methanol: water (70:30, v/v). The mobile phase was degassed by a degasser before pumped at flow rate 1 ml/min. The injected volume of the standard solution was 20 µl and UV detection at 260 nm.

Linearity

Aliquots of standard SFB solution (100 µg/ml) containing (50–350 µg) of intact SFB were transferred into a series of 10 ml volumetric flasks containing (150 µg) of atorvastatin (internal standard) and adjusted to volume with mobile phase. Into HPLC column, 20 µl were injected from each concentration under the described chromatographic conditions. Calibration graph was constructed by plotting the peak area ratio against the corresponding drug concentration in µg/ml and the regression equation was derived.

Ratio methods

Different aliquots of SFB standard solution ranging from (50–350) µg were transferred to a 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank, and then divided by the spectrum of SFB degradates solution (30 µg/ml).

A. Ratio difference method

The difference in the peak amplitudes (ΔP) at the ratio spectra was measured at 270 and 245 nm ($\Delta P_{270\text{-}245\ nm}$). The measured ΔP values versus the final concentrations in µg/ml were plotted to get the calibration graph and the regression equation was derived.

B. First derivative of ratio spectra method

The first derivative corresponding to each ratio spectrum was recorded, using $\Delta\lambda$ = 8 nm. The amplitude values at 282 nm were measured. The measured amplitude

values versus the final concentrations in μg/ml were plotted to get the calibration graph and the regression equation was derived.

C. Mean centering of ratio spectra method

The ratio spectra (from 200 to 400 nm) were mean centered and the mean centered values were measured at 262.6 nm. The measured mean centered values versus the final concentrations in μg/ml were plotted to get the calibration graph and the regression equation was derived.

Analysis of pharmaceutical preparation

Five tablets of SOVALDI® 400 mg were weighed and finely powdered. An accurately weighed amount of the powder equivalents to 10 mg was dissolved in methanol, filtered into 100 ml volumetric flask and the volume was completed to volume with methanol to obtain a solution labeled to contain 100 μg/ml of SFB. Transfer aliquots covering the working concentration range into 10 ml volumetric flasks. Proceed as described under "General Procedure" for each method. Determine the content of the tablets either from the calibration curve or using the corresponding regression equation.

RESULTS AND DISCUSSION

Degradation of SFB

Accelerated degradation method of SFB was achieved upon heating under reflux with 1 M sodium hydroxide for 38 hours. Later on, complete degradation of SFB was confirmed by TLC. For this purpose, the solution after reflux with 1M sodium hydroxide for 38 hours was cooled, neutralized with 1 M hydrochloric acid, evaporated under vacuum till dryness, extracted with methanol and filtered. The obtained solution was tested by TLC on silica gel 60 GF_{254} plates. Separation of the intact drug and its corresponding degradate was achieved by using mobile phase consists of methanol – chloroform – glacial acetic acid (50: 50: 0.1 by volume) and UV detection at 254 nm. R_f values of the intact SFB and its corresponding degradate were 0.26 and 0.61, respectively.

HPLC method

In the present study, a simple and sensitive reversed phase HPLC procedure was suggested for the selective quantitative determination of SFB in presence of its alkaline degradation product.

Different chromatographic conditions affecting the separation were tested taking in consideration the resolution between the drug, its degradation product and the internal standard. Several mobile phases were tried in order to separate the intact drug from its degradate and the internal standard including methanol: water in different ratios. Good separation was carried out on Athena C18 (250 X 4.6 X 5μm particle size) column using a mobile phase consists of methanol: water (70:30, v/v) at flow rate 1 ml min^{-1} and UV detection at 260 nm.

In HPLC chromatogram, showed in Figure 2, the peak of intact SFB, its degradation product and the internal standard were clearly separated and their corresponding peaks were sharply developed at reasonable retention times of 2.8 ± 0.02, 4.8 ± 0.03 and 6.9 ± 0.03 minutes for intact SFB, atorvastatin (internal standard) and degradation product respectively.

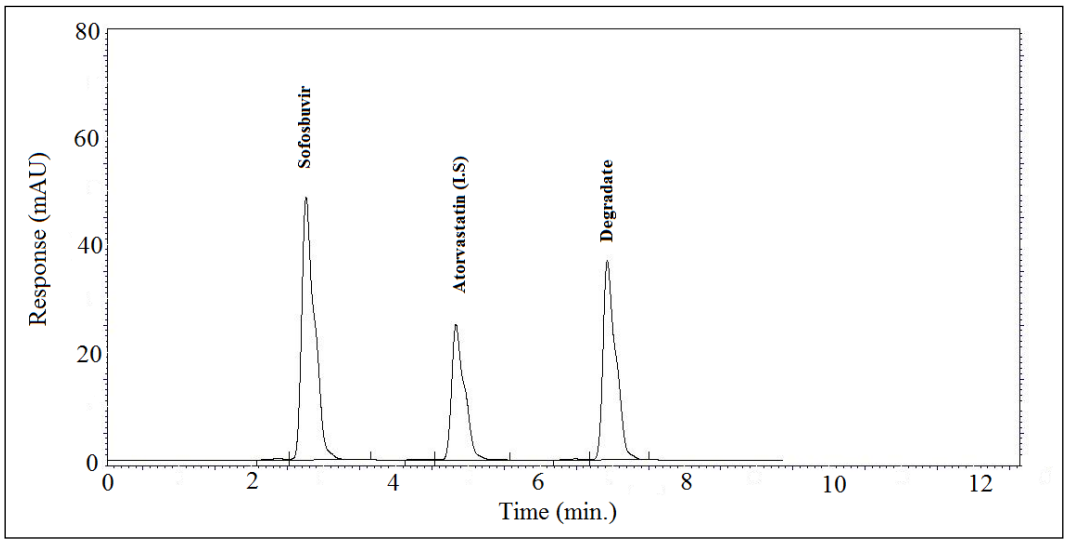

Figure 2. HPLC chromatogram of a mixture of intact SFB (35 µg/ml), atorvastatin as internal standard (15 µg/ml) and degradation product (30 µg/ml).

Ratio difference method

The zero-order absorption spectra of SFB and its alkaline degradate (Fig. 3) show severe overlap, which does not permit direct determination of SFB in presence of its degradate.

In this method, the absorption spectra of SFB were divided by a suitable absorption spectrum of its degradation product as a divisor to get the ratio spectra. The difference in peak amplitudes between two selected wavelengths in the ratio spectra was found to be proportional with the concentration of the drug without interference from its degradation product (Fig. 4). The method comprises two critical steps, the first is the choice of the divisor; the selected divisor should compromise between minimal noise and maximum sensitivity. The divisor concentrations of 30 µg /ml gave the best results. The second critical step is the choice of the wavelengths at which measurements are to be recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and give good linearity at each wavelength individually. The best results were obtained at 245 and 270 nm ($\Delta P_{270\text{-}245\ nm}$).

First derivative of ratio spectra method

In this method, the absorption spectra of SFB were divided by a suitable absorption spectrum of its degradation product as a divisor to get the ratio spectra. By application of the first- derivative to these ratio spectra, SFB can be quantitatively determined at 282 nm without any interference from its degradation product (Figs. 5, 6).

Careful choice of the divisor and the working wavelength were of great importance. The divisor concentration of 30 µg /ml was found to be the best. It produces minimum noise and gives better results in accordance with selectivity.

Mean centering of ratio spectra method

In this method, the absorption spectra of SFB were divided by a suitable absorption spectrum of its degradation product as a divisor to get the ratio spectra. The obtained ratio spectra were mean centered. The mean centered values at 262.6 nm was found to be proportional with the concentrations of the drug without interference from its degradation product (fig. 7). Careful choice of the divisor concentration was of great importance. The divisor concentrations of 30 µg /ml gave the best results in accordance with selectivity.

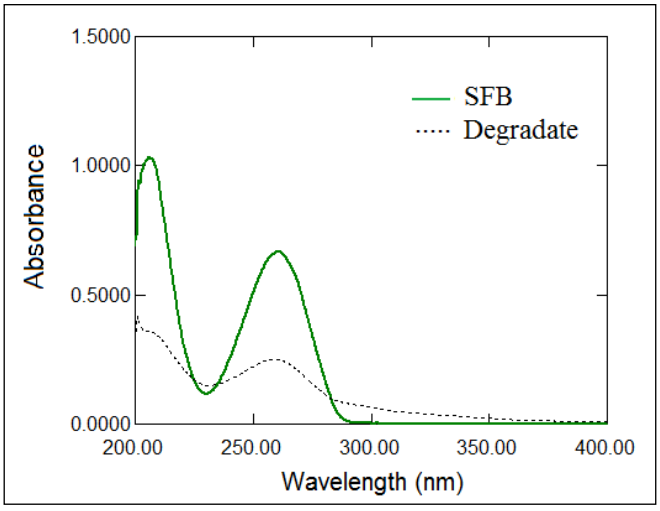

Figure (3): Absorption spectra of SFB (30 µg/ ml) and its alkaline degradate (30 µg/ ml).

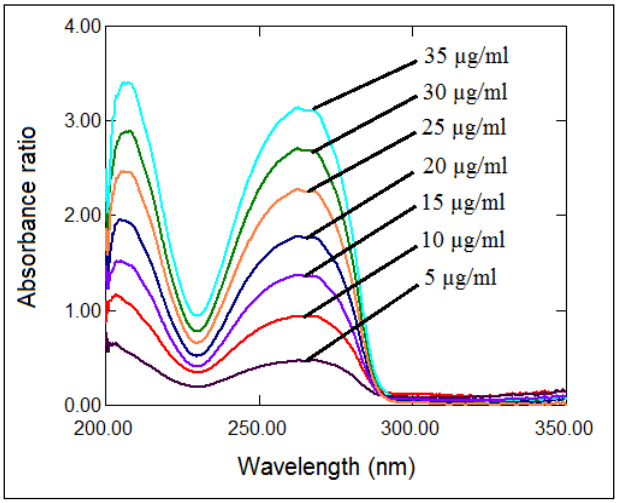

Figure (4): Ratio spectra of SFB at various concentrations using 30 µg/ml of alkaline degradate as a divisor.

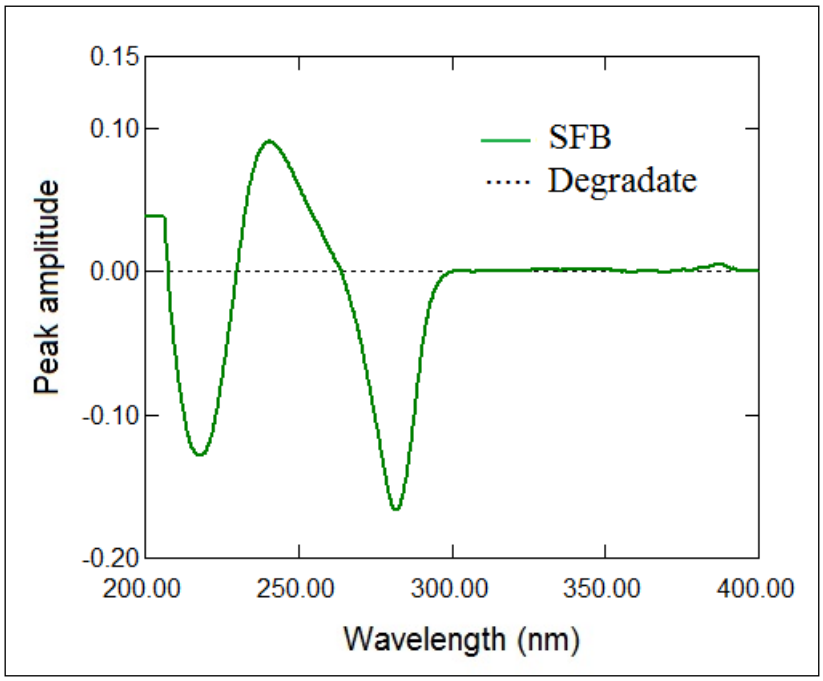

Figure (5): First derivative of the ratio spectra of SFB (30 µg/ml) and its alkaline degradate (30 µg/ml) using 30 µg/ml of degradate as a divisor.

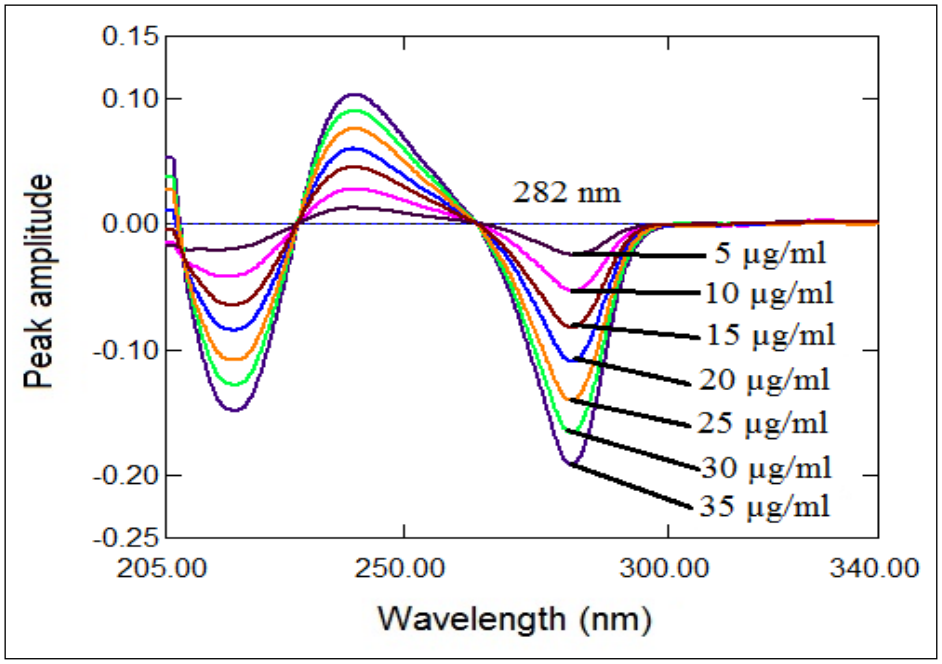

Figure (6): First derivative of the ratio spectra of SFB at various concentrations using 30 µg/ml of degradate as a divisor.

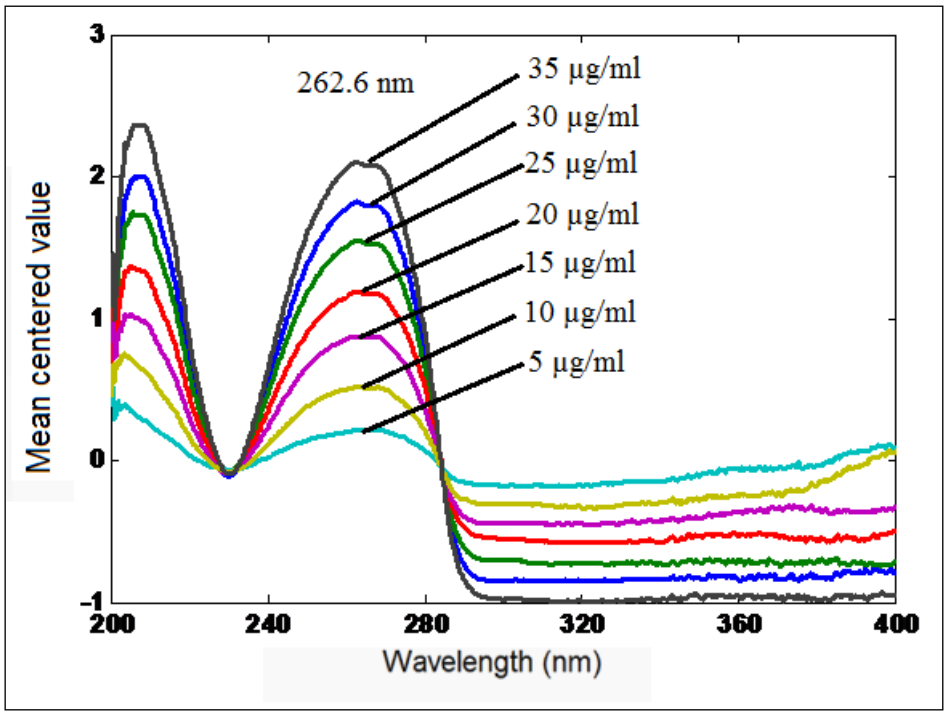

Figure (7): Mean centering of the ratio spectra of SFB at various concentrations using 30 µg/ml of degradate as a divisor.

Validation of the methods (ICH, 2005)

Linearity and range

Under the described experimental conditions, the calibration graphs for the methods were constructed by plotting the response versus drug concentrations in µg/ml. The regression plots were found to be linear over the range of 5-35 µg/ml for the four methods. Linearity ranges, regression equations, intercepts, slopes and correlation coefficients for the calibration were listed in **Table 1**.

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the following equations*:*

$$LOD = 3.3\ \sigma / S$$

$$LOD = 10\ \sigma / S$$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

The small values of LOD and LOQ **(Table 1)** indicate good sensitivity.

Accuracy and precision

Three replicate determinations of three different concentrations of SFB in pure form within linearity range for each method were performed in the same day (intra-day)

and in three successive days (inter-day). Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated **(Table 2)**. The small values of RSD% indicate high precision of the methods. Morever, the good R% confirms excellent accuracy.

Specificity

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the intact drug together with its degradation product. The proposed methods were adopted for the specific determination of intact SFB in presence of up to 86% of its degradate with mean recoveries of 100.66±1.310, 101.04±1.662, 101.06±1.026 and 99.92±1.374 for HPLC, ratio difference, ratio derivative and mean centering methods respectively **(Table 3)**.

System suitability

System suitability test for HPLC method was applied to a representative chromatogram to check various parameters such as the number of theoretical plates (N), resolution factor (R), capacity factor (K^-), tailing factor (T) and selectivity factor(α). The results obtained revealed that the chromatographic conditions described here allow complete base line separation between drug, its degradate and the internal standard peaks with minimum tailing.

Robustness

The robustness of the HPLC method was evaluated by slight changes in the chromatographic conditions such as flow rate (±0.1 ml/min.) and mobile phase contents ratio (±3%). It was found that; these minor changes did not affect the system suitability parameters, confirming robustness of the procedure.

The robustness of the three ratio methods was evaluated by slight changes in the optimum conditions such as divisor concentration (±0.2 µg/ml) and wavelength at which measurements done (±0.2 nm). It was found that; these minor changes did not affect the results, confirming robustness of the procedures.

Stability of standard solutions

The stability of standard solutions of SFB and the internal standard (atorvastatin) were determined by repeated analysis of solutions stored either at room temperature or in refrigerator at different time intervals and comparing the responses (peak areas) with those of freshly prepared standard solutions**.** From the results, it was found that, both SFB and atorvastatin standard solutions were stable for at least 3 and 7 days when stored at room temperature and in refrigerator, respectively.

Pharmaceutical Applications

The proposed methods were applied to the determination of SFB in **SOVALDI® tablets**. The results were validated by comparison to a previously reported method **(Al-Andalous Company)**. No significant difference was found by applying t-test and F-test at 95% confidence level **(Armitage, 1994)**, indicating good accuracy

and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form **(Table 4)**.

Table (1): Spectral data for determination of SFB by the proposed methods:

Parameters	**HPLC**	**Ratio difference**	**Ratio derivative**	**Mean centering**
Wavelength (nm)	260	270 and 245	282	262.6
Linearity range (µgml^{-1})	5 — 35	5 — 35	5 — 35	5 — 35
LOD (µgml^{-1})	0.534	0.665	0.555	0.499
LOQ (µgml^{-1})	1.620	2.014	1.680	1.511
Regression equation*				
Slope (*b*)	0.0654	0.0235	0.0057	0.0647
Intercept (*a*)	0.0268	-0.0014	-0.0035	-0.1103
Correlation coefficient (r^2)	0.9997	0.9996	0.9997	0.9998

* $y = a + bx$ where y is the response and x is the concentration in µgml^{-1}.

Table (2): Intraday and interday accuracy and precision for the determination of SFB by the proposed methods:

Method	Conc µg.ml^{-1}	Intraday			Interday		
		Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
HPLC	10	10.13 ± 0.054	101.31	0.536	10.04 ± 0.098	100.41	0.978
	20	20.12 ± 0.241	100.62	1.200	20.09 ± 0.257	100.44	1.277
	30	29.81 ± 0.274	99.37	0.920	29.79 ± 0.128	99.30	0.428
Ratio difference	10	9.99 ±0.085	99.94	0.849	10.00 ±0.077	99.97	0.770
	20	20.01 ±0.217	100.04	1.085	20.05 ±0.236	100.25	1.179
	30	30.28 ±0.209	100.93	0.689	30.18 ±0.337	100.58	1.116
Ratio derivative	10	10.06 ± 0.095	100.62	0.944	10.04 ± 0.101	100.37	1.007
	20	20.14 ± 0.259	100.68	1.286	20.11 ± 0.245	100.56	1.217
	30	29.94 ± 0.302	99.80	1.008	30.11± 0.229	100.38	0.760
Mean centering	10	9.84 ± 0.080	98.39	0.812	9.83 ± 0.090	98.33	0.914
	20	20.02 ± 0.159	100.09	0.792	20.05 ± 0.208	100.25	1.036
	30	30.01 ± 0.286	100.04	0.953	30.12 ± 0.286	100.39	0.889

Table (3): Determination of intact SFB in mixtures with its alkaline degradate by the proposed methods:

Method	Intact (µg ml^{-1})	Degradate (µg ml^{-1})	Degradate %	Intact found (µg ml^{-1})	Recovery % of Intact
HPLC	30	5	14	29.57	98.57
	25	10	29	25.00	100.00
	20	15	43	20.03	100.14
	15	20	57	15.30	101.99
	10	25	71	10.15	101.53
	5	30	86	5.09	101.71
	Mean ± RSD%				100.66±1.310
Ratio difference	30	5	14	30.51	101.71
	25	10	29	25.22	100.89
	20	15	43	20.33	101.64
	15	20	57	15.29	101.94
	10	25	71	10.23	102.30
	5	30	86	4.89	97.74
	Mean ± RSD%				101.04±1.662
Ratio derivative	30	5	14	30.24	100.80
	25	10	29	25.11	100.44
	20	15	43	20.31	101.55
	15	20	57	15.21	101.40
	10	25	71	10.20	102.00
	5	30	86	5.17	103.40
	Mean ± RSD%				101.06±1.026
Mean centering	30	5	14	30.18	100.60
	25	10	29	25.35	101.40
	20	15	43	20.18	100.90
	15	20	57	14.95	99.70
	10	25	71	9.93	99.30
	5	30	86	4.97	100.60
	Mean ± RSD%				99.92±1.374

Table (4): Determination of SFB in **SOVALDI®** tablets by the proposed and reported methods:

Parameters	HPLC	Ratio difference	Ratio derivative	Mean centering	Reported method**
N*	5	5	5	5	5
$\bar{X}$	100.21	100.29	100.38	100.16	100.04
SD	1.248	0.780	0.838	0.684	1.037
RSD%	1.245	0.777	0.835	0.683	1.037
t***	0.23 (2.31)	0.42 (2.31)	0.57 (2.31)	0.21 (2.31)	———
F***	1.45 (6.39)	1.77 (6.39)	1.53 (6.39)	2.30 (6.39)	———

** No. of experimental.*

*** It is an HPLC method using Zorbax phenyl (250 X 4.6 X 5µm particle size) column and phosphate buffer pH 3.5: acetonitrile (60:40, v/v) as mobile phase at flow rate 1.5 ml/min and UV detection at 260 nm* ***(Al-AndalousCompany)*** *.*

**** The values in the parenthesis are tabulated values of t and F at (p= 0.05).*

CONCLUSION

Simple, rapid, sensitive, accurate, precise and not expensive methods were developed for the analysis of SFB in pure form, in its tablets form and in presence of its alkaline degradation product. The sensitivity, reproducibility and simplicity of the proposed methods make them valuable in routine analysis of SFB. In addition, the proposed methods were found to be stability indicating methods.

ACKNOWLEDGMENT

I am deeply thankful to **ALLAH**, by the grace of whom this work was realized. I wish to express my indebtedness and gratitude to staff members Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their co-operation throughout the whole work.

REFERENCES

Abdallah, O. M; and Badawey, A. M. (2011): Int. J. Ind. Chem., 2(2): 78-85.

Afkhami, A; and Bahram, M. **(2004):** Anal. Chim. Acta, 526(2): 211-218.

Afkhami, A; and Bahram, M. (2005): Talanta, 66(3): 712-720.

Afkhami, A; and Bahram M. (2006): Talanta, 68(4): 1148-1155.

Al-Andalous Company: Quality control and quality assurance units "Al-Andalous for Pharmaceutical Industries Company, Giza, Egypt". (Personal communications)

Armitage, P; and Berry, G. (1994) : Statistical Methods in Medical Research. 3rd ed. Oxford, UK; Blackwell

Belal, T. S; Daabees, H. G; Abdel-Khalek, M. M; Mahrous, M. S; and Khamis, M. M. (2013): J. Pharm. Anal., 3(2): 118-126.

British Pharmacopoeia (2012): The Stationery Office, Vol. 5. London, UK.

Connors, A. K (2005): A Text book of pharmaceutical analysis. 3rd ed. Hoboken (NJ): Wiley Interscience Publication.

Darwish, H. W; Hassan, S. A; Salem, M. Y; and El-Zeany, B. A. (2013): Spectrochimca Acta Part A: Mol. Biomol. Spectrosc., 104: 70-76.

Darwish, H.W; Hassan, S.A; Salem, M.Y; and El-Zeiny, B. A. (2011): Spectrochimca Acta Part A: Mol. Biomol. Spectrosc., 83(1): 140-148.

EL-Bagary, R. I; Abo-talib, N.F; and Nor-Eldin, M. B. (2011): J. Chem. Pharm. Re., 3(6): 562-570.

El-Ragehy, N. A; Abbas, S. S; and El-Khateeb, S. Z. (2002): Anal. Chim. Acta., 461: 155–168.

Elzanfaly, E. S; Saad, A. S; Abd Elaleem, A. B. (2012): J. Pharm. Anal., 2(5): 382-385.

Harvey, D. (2000): Modern analytical chemistry. New York: McGraw-Hill.

ICH (2005): Validation of analytical procedure. Text and methodology. Geneva: International conference on Harmonization.

Issa, Y. M; Zayed, S. M; and Habib, I. I. (2011): Arabian J. Chem., 4: 259-263.

Lotfy, H. M; and Hagazy, M. A. (2012): Spectrochimca Acta Part A: Mol. Biomol. Spectros., 96: 259-270.

Lotfy, H. M; Saleh, S. S; Hassan, N. Y; and Elgizawy, S. M. (2012): J. Anal. Chem., 3: 761-769.

Martin, M; and Guiochon, G. (2005): J Chromatogr A., 1090(1-2): 16-38.

RxList (2015): the internet drug index [Internet]. California: WebMD; 2004. Sovaldi; [reviewed 2015 Jun 4; cited 2015 Jun 27]. Available from: http://www.rxlist.com/sovaldi-drug.htm

Willard, H. H; Dean, A. J. (1986): Instrumental Methods of Analysis. 7th ed. New Delhi: CBS Publishers and Distributors.

الملخص العربى

استخدام كروماتوجرافيا السوائل ذات الاداء العالي والمطياف الضوئي لتعيين ثباتية عقار السوفوسبفير سواء في صورته النقية او شكله الصيدلي

للسيد الدكتور

احمد عبد الحليم ابوسريع محمد

مـــــــــــــــن

قسم الكيمياء التحليلية كلية الصيدلة جامعة الازهر بالقاهرة

فى هذا البحث تم استنباط اربعة طرائق - بسيطه وحساسه وعلى درجة عاليه من الدقه - داله على الثباتيه لتعيين مادة السوفوسبفير سواء فى صورتة النقية أو شكله الصيدلى أوفى وجود ناتج تكسيره القلوى. الطريقة الاولى تم خلالها تعيين مادة السوفوسبفير فى وجود ناتج التكسير القلوى باستخدام كروماتوجرافيا السوائل ذات الأداء العالى وقد تم التوصل الى طريقة لفصل الدواء عن ناتج التكسير القلوى فى وجود أتروفاستاتين (مادة عيارية داخلية) باستخدام عمود الطبقة المعكوسة وسائل متحرك مكون من الميثانول - الماء (٧٠ : ٣٠ حجم/ حجم) ومعدل تدفق ١ مل لكل دقيقة مع قياس نواتج الفصل عند موجة ضوئية طولها ٢٦٠ ن.م. الطريقة الثانية تم خلالها تعيين مادة السوفوسبفير فى وجود ناتج التكسير القلوى باستخدام فرق النسبة الدالة على الثبات وذلك عند طوليين موجيين ٢٤٥ و ٢٧٠ ن.م. الطريقة الثالثة تم خلالها تعيين مادة السوفوسبفير فى وجود ناتج التكسير القلوى باستخدام المقياس الطيفى للمشتق التفاضلى الأول وذلك عند طول موجى ٢٨٢ ن.م. الطريقة الرابعة تم خلالها تعيين مادة السوفوسبفير فى وجود ناتج التكسير القلوى باستخدام حساب المتوسط المركزى الدال على الثبات عند طول موجى ٢٦٢.٦ ن.م. وقد تم الحصول على علاقة طردية مباشرة فى مدى تركيز قدره ٥ – ٣٥ مكجم / مل من السوفوسبفير. وقد اظهرت الطرائق انتقائية لمادة السوفوسبفير فى وجود ٨٦ % من ناتج التكسير القلوى وقد تم الحصول على نتائج دقيقة فى الإسترجاع حيث كانت ١٠٠.٦٦ ± ١.٣١٠ و ١٠١.٠٤ ± ١.٦٦٢ و ١٠١.٠٦ ± ١.٠٢٦ و ٩٩.٩٢ ± ١.٣٧٤ % للاربع طرائق على التوالى. وقد تم تطبيق هذه الطرائق بنجاح لتعيين مادة السوفوسبفير كمادة خام وفى المستحضر الصيدلى كما تم عمل مقارنة احصائية لنتائج الطرائق المستنبطة ونتائج الطريقة المرجعية واتضح انه لا يوجد فرق من حيث الدقة والضبط.