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Original Article

Simultaneous separation of antihyperlipidemic drugs by *green* ultrahigh-performance liquid chromatography–diode array detector method: Improving the health of liquid chromatography

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ABSTRACT

Statins in combination with fibrates show beneficial effects on the lipoprotein profile of patients because they have positive complimentary effects on lipid profile. A new *green* ultrahigh-performance liquid chromatography–diode array detector method for simultaneous analysis of simvastatin (SMV) and fenofibrate (FNF) in standard form, marketed formulations, and self-emulsifying drug delivery system formulations was developed and validated in the present investigation. The method utilized C_{18} as stationary phase and a combination of methanol:water (8:2) as an eluent. It was found that selected eluent provided short run time (2.5 minutes), better peak symmetry and satisfactory values of other chromatographic parameters such as resolution ($Rs = 2.325$), capacity factor (k, 3.0 and 4.2 for SMV and FNF, respectively), selectivity ($\alpha = 1.4$), and number of theoretical plates (N, 4265 and 5285 for SMV and FNF, respectively). An excellent linear relationship (r^2 0.998 and 0.997 for SMV and FNF, respectively) was observed for linear regression data for the calibration plots. The developed system was validated for accuracy, precision, robustness (< 2% for both drugs) and recovery (98–102% for both drugs). Results obtained from the statistical treatment of the values obtained for different parameters proved that the method is suitable, reproducible, and selective for the simultaneous analysis of SMV and FNF in bulk, marketed, and self-emulsifying drug delivery system formulations. The replacement of commonly applied toxic solvents with innocuous and environmentally benign solvents provides a better option than the more toxic processes in drug analysis.

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1. Introduction

Hyperlipidemia, characterized by the presence of an increased lipid concentration in the blood is one of the indicative factor (combined with other factors such as hyperglycemia, obesity, high blood pressure, and defective fibrinolysis) for cardiovascular disease. It is also a potential factor for the development of atherosclerosis in diabetes mellitus [1–3]. Co-administration of statins and fibrates have been suggested by The National Cholesterol Education Program Adult Treatment Panel III for the management of patients with hyperlipidemia

Statins in combination with fibrates show beneficial effects on the lipoprotein profile of patients with combined hyperlipidemia, and this is well accepted with a safety profile the same as individual monotherapies [4,5]. Chemically, statins are 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors that possess the property of inhibiting the production rate of cholesterol in the body [6]. Fibrates have been found to decrease the production and elevate the rate of breakdown of cholesterol and triglycerides in the body by promoting β-oxidation of fatty acids primarily in the peroxisomes and partly in mitochondria [7,8]. Both statins and fibrates have been found to work through different pathways and have positive complimentary effects on the lipid profile of patients [9]. Due to their inherent property of exhibiting poor water solubility and low absorption after oral administration, both simvastatin (SMV) and fenofibrate (FNF) are considered to be Class II drugs in the Biopharmaceutical Classification System. Limited solubility in physiological fluids combined with low permeability through the gastrointestinal membrane limits *in vivo* absorption and thus bioavailability of such drugs, which is a hindrance in the development of suitable dosage forms [10]. Many different formulation strategies have been proposed and practiced for improving the solubility and bioavailability of hydrophobic compounds such as complexation with β-cyclodextrin or caffeine, salt formation, conjugation to dendrimers and use of cosolvents have been employed to solubilize hydrophobic compounds.

Lipid-based drug delivery systems are one of the most popular approaches in the field of drug delivery with range from simple solutions or suspensions of drugs in lipids to complex mixtures of oils, surfactants, cosurfactants and cosolvents. Many of these mixtures are characterized as self-emulsifying drug delivery systems (SEDDS) [11,12]. In order to overcome the challenge of increasing bioavailability of hydrophobic drugs, SEDDS is a promising technique that keeps the drug molecule in solubilized form in the tiny droplets of oil (in a mixture of surfactant and oil), as a result of which greater interfacial surface area becomes available for enhanced absorption of drug molecule. This mixture has the ability of forming oil-in-water emulsions when its gets shaken by gastrointestinal movements [13]. In addition, lipid as a constituent of the formulation also plays its part in improving bioavailability by increasing drug absorption.

Many analytical techniques are used for the determination of statins or FNF in standard form, developed formulations, and plasma as well as in *in-vivo* and *in-vitro* studies [14,15]. However, a literature survey of recent years revealed that few studies were performed for the simultaneous determination of statin and fibrates irrespective of the fact that it is preferable to prescribe coadministration of statins with fibrates for patients with dyslipidemia [16].

Ultrahigh-performance liquid chromatography (UHPLC) is a relatively new and advanced liquid chromatographic technique that makes the resolution possible in significantly less time because of very fine particle size columns (approx. 3 μm) with significantly lower consumption of eluents [17]. Atorvastatin and its pharmaceutical formulation with FNF is not official in any pharmacopoeia yet. As a result, there are very few reports on the simultaneous analysis of SMV and FNF in the literature. This study was undertaken with the aim of developing a UHPLC–diode array detector (DAD) method for the concurrent analysis of SMV and FNF in standard form and marketed formulations. Derivative ratio spectrophotometry and chemometric calibrations method has been described for simultaneous separation of atorvastatin and FNF [18–20].

In spite of their environmentally adverse effects, the most commonly used solvents as mobile phase in HPLC are volatile organic solvents such as acetonitrile. These solvents require special treatment before being discharged into the water bodies or land. Among the principles of *green* chemistry, there is great emphasis for promoting the use of alternative solvents and auxiliaries to decrease the adverse environmental impact of toxic solvents [21]. Literature review over 15 years clearly indicates the elevated use of environmentally safer solvents [22–25]. Capello et al [26,27] proposed a concept for the environmental impact of solvents that is based on the application of two environmental evaluation methods with varying scopes. The first is the environmental, health, and safety assessment method [28] that evaluates the potential hazards of chemicals. The other method, the life-cycle assessment method, was employed for a complete assessment of releases to the environment as well as resource use over the full life-cycle of a solvent. Based on this study, to our surprise, it was found that methanol–water mixture was environmentally favorable in all proportions and this acceptability increases with increasing water content. Therefore, the mixture of methanol and water was put to test for the analysis of SMV and FNF (Figure 1) in bulk drug, marketed products, and laboratory-prepared SEDDS formulation by UHPLC-DAD technique. The developed method was capable to resolve these compounds with a run time under 2 minutes. The effect of constituents of SEDDS in the analysis of SMV and FNF was also performed to check the possible interference.

2. Materials and methods

2.1. Chemicals and reagents

SMV (purity 99.9%) was purchased from Riyadh Pharma (Riyadh, Saudi Arabia). FNF (purity 99.99%) was purchased from Sigma–Aldrich (St Louis, MO, USA). HPLC grade methanol was procured from BDH Laboratory supplies (Liverpool, UK). All other reagents and chemicals employed were of analytical reagent quality. Commercial tablets—lipanthyl 200M (Solvay Pharmaceuticals, Marietta, GA, USA) for FNF and Zocor 10 mg for SMV (Merck, Sharp & Dohme, Kenilworth, NJ,

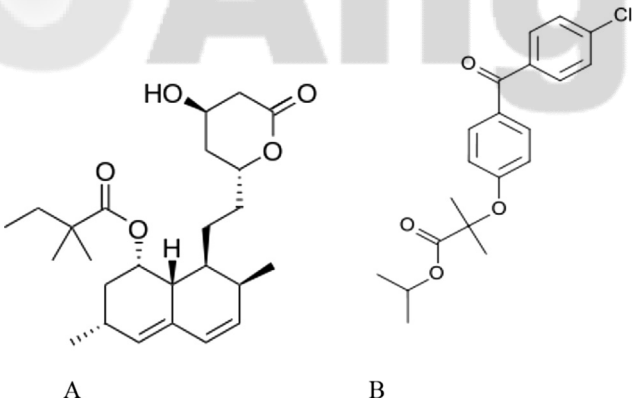

Figure 1 – Structures of (A) simvastatin and (B) fenofibrate.

USA)—were obtained from the local market of Riyadh, Saudi Arabia. SMV and FNF SEDDS was prepared in the laboratory by the spontaneous emulsification method using Imwitor 308 (I308, 98% monocaprylate) as the oil phase, cremophor EL as surfactant, transcutol as cosurfactant and distilled water as an aqueous phase.

2.2. *Instrumentation and chromatographic conditions*

Simultaneous analysis of SMV and FNF was done at room temperature (22 ± 1°C), with Thermo Scientific UHPLC system (Thermo Scientific, Karlsruhe, Germany) provided with a 3000 LC pump, 3000 autosampler, binary pumps, a programmable DAD detector, ultimate 3000 column oven, ultimate 3000 controller, and an inline vacuum degasser, and based on Chromeleon software, version 6.8. Chromatography was performed on a Thermo Hypersil GOLD 50 × 2.1 mm reversed phase C18 column (Thermo Scientific) having a 1.9-μm size particle as static phase. The mobile phase consisted of methanol–water (80:20, v/v). The chromatography was done at a flow rate of 0.4 mL/min with DAD detection at 240 nm. Samples (1 μL) were introduced using an ultimate 3000 series Thermo auto sampler.

2.3. *Preparation of SMV and FNF stock solution*

Linearity plot for SMV and FNF was plotted in concentration range of 0.05–100 μg/mL. Working dilutions in the desired range were prepared from the stock solution (100 μg/mL) by suitably mixing the required aliquots with the mobile phase to get the desired concentration.

2.4. *Method development*

Various mixed organic/hydro-organic solvent systems as mobile phase were tried for the development of suitable UHPLC–DAD method for the simultaneous quantification of SMV and FNF in its standard drug compound. Various parameters were kept in mind while deciding the suitability of any solvent or mixture of solvents to be used as mobile phase such as appropriateness for stability studies, sensitivity of the developed method, time consumed for the analysis, peak parameters, mutual miscibility of the constituent solvents, and the economy of the solvents. Based on the above criteria, various mobile phases such as methanol–water, methanol–phosphate buffer, acetonitrile–phosphate buffer, methanol–sodium percholate buffer, and acetonitrile–sodium percholate buffer in varying proportions were tried. Among the various tried solvents systems for UHPLC quantification, a combination of methanol–water (80:20, v/v) was selected as most suitable eluent for subsequent studies.

2.5. *Validation studies*

The newly developed UHPLC–DAD method was validated for various parameters such as linearity, precision, accuracy, robustness, sensitivity and specificity [29,30]. Freshly made dilutions in the concentration range of 0.05–100 μg/mL were used for plotting of linearity curves. The solvent system consisting of methanol–water (80:20, v/v) was dispatched at 0.4 mL/min for bringing the column to equilibrium and the baseline was continuously observed and monitored during the entire process. The drugs SMV and FNF were detected at 240 nm. The freshly prepared dilute solutions were introduced into the system in the multiples of three and peak areas were recorded using the UHPLC system for each solution and calibration was obtained by plotting concentration vs. peak area.

Accuracy of the developed method was assessed by previously reported standard addition method. The standard target SMV and FNF solution (10 μg/mL) was mixed with 0%, 50%, 100%, and 150% surplus drug standard solution and reanalyzed by the newly developed UHPLC–DAD method. Each experiment was done in triplicate. Percent recovery, standard deviation (SD), and standard error for each concentration were calculated.

Precision of the newly developed UHPLC–DAD method was estimated at two different levels, i.e. repeatability (intraday precision) and interday (intermediate) precision. Intraday precision of the proposed UHPLC–DAD method was carried out by quantification of five different concentrations of SMV and FNF (5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL) three times on the same day. However, intermediate precision of the proposed UHPLC–DAD method was determined by reanalyzing the samples on three different days.

The robustness of the proposed UHPLC–DAD method was determined to evaluate the effect of deliberate changes made in the chromatographic conditions on the analysis of SMV and FNF. The concentration (10 μg/mL) of SMV and FNF was selected for this purpose. Robustness of the proposed UHPLC–DAD method was evaluated by slightly increasing as well as decreasing the mobile phase flow rate from 0.4 mL/min to 0.5 mL/min and 0.3 mL/min, wavelength of detection from 240 nm to 250 nm and 270 nm, and by varying the ratio of methanol:water in mobile phase from 80% to 90% and 70%.

2.6. *Marketed product analysis*

Commercially available tablets were crushed to fine powder. An accurately weighed portion of the powder equivalent to 5 mg, 25 mg, and 50 mg of both SMV and FNF was taken together in 50 mL volumetric flask. Methanol (about 30 mL) was added to the flask followed by sonication in an ultrasonic bath for 3 minutes. After sonication, about 20 mL methanol

was further added to make up 50 mL and sonicated for 5 minutes. The solution was then filtered using 0.45 μm nylon filter and, after discarding the first few mL, filtrate was collected. Five mL of this filtrate was transferred to 50 mL volumetric flask, diluted to volume with diluent and stirred suitably for proper mixing.

2.7. *Application of UHPLC–DAD method for the simultaneous assay SMV and FNF in SEDDS*

The validity of UHPLC–DAD method was identified by applying it for the assay of SMV and FNF in SEDDS. SEDDS formulation of SMV and FNF was prepared in the laboratory by spontaneous emulsification method using Imwitor 308 (I308, 98% monocaprylate) as the oil phase, cremophor EL as surfactant, Transcutol as cosurfactant, and demineralized water as the aqueous phase. To analyze the amount of SMV and FNF in prepared SEDDS (having 5 μg/mL each of SMV and FNF: single dose), 1 mL of SEDDS was properly diluted with eluent to make 100 mL of stock solution, which was subsequently sonicated for 10 minutes and then evaluated for the amount of SMV and FNF present in SEDDS. [27]. The prepared stock solution was then sonicated for about 1 hour and subjected to UHPLC–DAD analysis for determination of SMV and FNF content in the formulation. The interactions between SMV and FNF and nanoemulsion components were also investigated by the proposed method.

3. Results and discussion

3.1. *Chromatography and method validation*

The important criteria for development of successful UHPLC method for analysis of SMV and FNF in bulk drug and in marketed formulations were: the method is expected to be able to resolve satisfactorily both drugs simultaneously and as well as be accurate, reproducible and robust. It should also be able to analyze drugs in the presence of SEDDS components and simple enough for routine use in quality control laboratory.

During the method development step, use of methanol and phosphate buffer (component of phosphate buffer (pH 7) was monobasic potassium phosphate and sodium hydroxide) as the mobile phase produced asymmetric peak with a larger tailing factor (> 2) and fewer number of theoretical plates (< 2000). Further, acetonitrile was tried in combination of phosphate buffer at different proportions at flow rate of 0.5 mL/min. The chromatogram was obtained with unsatisfactory peak parameters. In yet another attempt to get a satisfactory peak having asymmetry factor ≤ 2 and good sensitivity, methanol and water were tried as another eluting phase. Various compositions of methanol and water were tested, and the binary proportion at 80%:20% v/v was found superior with a sharp peak, appropriate retention time and fine asymmetry. Thus, methanol and water at 80:20 was used to develop a facile and quick method for SMV and FNF with a reasonable run time (2.50 minutes), appropriate retention time (2.093 ± 0.01 minutes for FNF and 1.613 ± 0.01 minutes for SMV) and the acceptable tailing or asymmetry factor (Figure 2). Chromatographic parameters such as resolution (*Rs*), capacity factor (*k*), peak symmetry, selectivity (*α*), and number of theoretical plates (*N*) were calculated for the obtained resolution showing satisfactory separation of the drugs (Table 1). The resolution of both compounds was studied using this mixture of methanol:water on UHPLC C_{18} column and system.

3.2. *Proposed mechanism of separation*

In principle, any separation on reverse-phase column is based on partition of analytes between the polar mobile phase and nonpolar stationary phase. Nonpolar (hydrophobic/

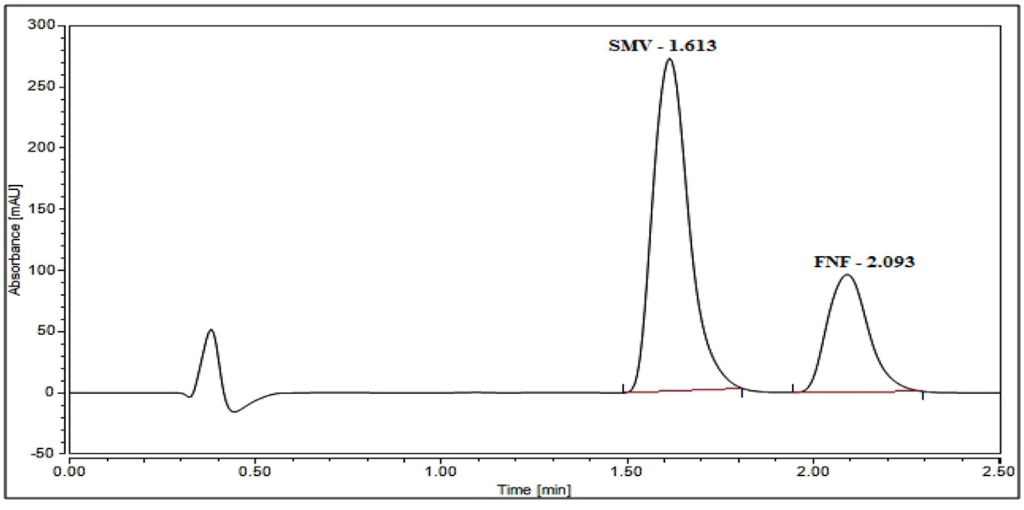

Figure 2 – Representative chromatogram showing separation of simvastatin (SMV) and fenofibrate (FNF). Retention times were: SMV 1.613 minutes and FNF 2.093 minutes. Column: Thermo Hypersil GOLD 50 mm × 2.1 mm reversed phase C_{18} column having a 1.9 μm size particle. Mobile phase: methanol–water (80:20, v/v); flow rate 0.4 mL/min; diode array detection at 240 nm. Analysis of SMV and FNF was done at room temperature (22 ± 1°C). The concentration of SMV and FNF was 10 μg/mL.

Table 1 – Chromatographic parameters for the separation of simvastatin (SMV) and fenofibrate (FNF).

Drug	Resolution (Rs)	Peak symmetry	Selectivity (α)	Capacity factor (k)	No. of theoretical plates (N)
SMV	2.32	0.9	1.4	3.0	4265
FNF		1.1		4.2	5285

nonspecific) interactions of SMV and FNF with hydrophobic (or lipophilic) stationary phase C_{18} results in different sorption affinities between the drugs, which promoted their separation. As SMV (topological polar surface area 72.8 Å^2) is more polar than FNF (topological polar surface area 52.6 Å^2), it shows more affinity towards the polar mobile as compared to its affinity towards nonpolar stationary phase, as a result of which it is less retained as compared to FNF.

3.3. *Validation of the method*

3.3.1. *Linearity*

The linearity of detector response to different concentrations of drugs was studied in the range of 0.05–100 μg/mL at eight different concentrations (0.05 μg/mL, 0.1 μg/mL, 0.5 μg/mL, 1 μg/mL, 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL). The samples were analyzed in triplicates at all concentrations. Calibration curves were constructed and found that correlation coefficient values of both the studied drugs were observed to be 0.997 for SMV and 0.998 for FNF respectively.

Table 2 – Linear regression data for calibration plots for simvastatin (SMV) and fenofibrate (FNF; $n = 3$).

Parameters	FNF	SMV
Linearity range (ng per spot)	0.05–100 mg/mL	0.05–100 mg/mL
Regression equation	$y = 0.525x + 0.252$	$y = 0.688x + 0.805$
Correlation coefficient	0.99	0.99
Slope ± SD	0.52 ± 0.02	0.68 ± 0.01
Confidence interval of slope	0.52–0.52	0.69–0.68
Intercept ± SD	0.25 ± 0.01	0.80 ± 0.01
Slope without intercept ± SD	0.52	0.70
Standard error of slope	0.30	0.39
Standard error of intercept	0.01	0.02
Confidence interval of intercept	0.25–0.24	0.80–0.80

SD = standard deviation.

The regression analysis data for calibration curves were calculated using the peak areas and the data are shown in Table 2.

3.3.2. *Precision*

The intraday precision (repeatability) of sample was evaluated as intraday variation whereas the intermediate precision was evaluated by measuring inter-day variation for simultaneous analysis of SMV and FNF at five different concentrations (5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL), in triplicate. Data obtained from determination of repeatability and intermediate precision expressed as %RSD are shown in Tables 3 and 4. The low magnitude of %RSD indicates the high repeatability of the method.

3.3.3. *Robustness*

The robustness of the developed method was determined by analyzing the samples after deliberate changes were made in the method parameters, such as flow rate, detection wavelength and composition of mobile phase. The low magnitude of %RSD obtained after introducing deliberate small changes in mobile phase composition indicates the robustness of the method. There was no significant variation of the slopes of the calibration plots (Table 5).

3.3.4. *Recovery*

The method employed for the estimation of SMV and FNF in pharmaceutical formulation after spiking with 50%, 100%, and 150% additional drug. The recovery was in the range of 98.71–101.7%, which suggests satisfactory accuracy of the method (Table 6).

3.4. *Analysis of SMV and FNF in marketed products*

Commercially available tablets of SMV and FNF were crushed and the powder equivalent to one tablet weight was weighed and diluted with methanol:water (8:2, v/v; or methanol) and sonicated for 15 minutes and further dilutions were made with mobile phase to obtain concentrations within the linearity range (5 μg/mL, 25 μg/mL, and 50 μg/mL of SMV *and* FNF, respectively). All the samples were filtered through a Whatman (GD/X 25, polypropylene, 0.45 μm) syringe filter, before

Table 3 – Intraday precision of the method ($n = 3$).

Concentration (μg/mL) FNF	Repeatability (intraday precision) FNF			Repeatability (intraday precision) SMV		
	Mean area ± SD (mAU*min)	RSD (%)	Standard error	Mean area ± SD	RSD (%)	Standard error
05	2.94 ± 0.03	1.36	0.02	3.15 ± 0.06	2.09	0.03
10	5.16 ± 0.14	2.76	0.08	6.16 ± 0.18	2.92	0.10
25	14.94 ± 0.10	0.72	0.06	15.17 ± 0.07	0.47	0.04
50	28.91 ± 0.07	0.24	0.04	30.02 ± 0.16	0.56	0.09
100	54.04 ± 0.24	0.45	0.01	59.31 ± 0.43	0.73	0.25

FNF = fenofibrate; RSD = relative standard deviation; SD = standard deviation; SMV = simvastatin.

Table 4 – Interday precision of the method ($n = 3$).

Concentration (μg/mL) SMV	Intermediate (interday precision) FNF			Intermediate (interday precision) SMV		
	Mean area ± SD (mAU*min)	RSD (%)	Standard error	Mean area ± SD	RSD (%)	Standard error
5	2.68 ± 0.08	3.20	0.04	3.64 ± 0.08	3.20	0.04
10	5.10 ± 0.09	1.92	0.05	6.70 ± 0.09	1.92	0.05
25	12.45 ± 0.37	3.02	0.21	16.62 ± 0.37	3.02	0.21
50	25.24 ± 0.21	0.84	0.12	33.64 ± 0.21	0.84	0.12
100	49.41 ± 0.14	0.29	0.08	65.16 ± 0.14	0.29	0.08

FNF = fenofibrate; RSD = relative standard deviation; SD = standard deviation; SMV = simvastatin.

Table 5 – Robustness of the method.

Parameters		FNF			SMV		
		Mean area ± SD (mAU*min)	RSD (%)	Standard error	Mean area ± SD	RSD (%)	Standard error
Mobile phase composition	9:1	2.76 ± 0.05	1.99	0.03	3.60 ± 0.05	1.45	0.03
	7:3	2.98 ± 0.03	1.15	0.01	3.64 ± 0.04	1.36	0.02
Mobile phase flow rate	0.5	2.83 ± 0.03	1.25	0.02	3.19 ± 0.04	1.29	0.02
	0.3	2.87 ± 0.02	1.02	0.01	3.17 ± 0.02	0.75	0.01
Detection wavelength (nm)	250	2.7 ± 0.03	1.19	0.01	3.71 ± 0.05	1.53	0.03
	270	2.9 ± 0.02	0.89	0.01	3.64 ± 0.02	0.55	0.01

FNF = fenofibrate; RSD = relative standard deviation; SD = standard deviation; SMV = simvastatin.

Table 6 – Results from recovery studies.

Excess drug added (%)	FNF				SMV		
	Total theoretical content	% Drug recovered	SD	Standard error	% Drug recovered	SD	Standard error
0	5.00	100.52	0.01	0.01	100.05	0.01	0.01
50	7.50	98.97	0.02	0.04	101.70	0.01	0.01
100	10.00	99.77	0.02	0.01	98.71	0.02	0.01
150	12.50	99.53	0.01	0.01	100.89	0.01	0.01

FNF = fenofibrate; SD = standard deviation; SMV = simvastatin.

placing the samples into the autosampler of UHPLC instrument. The data are shown in Table 6. All obtained values were within the range of ICH guidelines (98–102%). High assay value and low RSD (%) of both the drugs in over-the-counter drugs suggest that the method is suitable for routine analysis of FNF and SMV of over-the-counter commercially available drugs. The chromatogram of extracted FNF and SMV from the marketed tablets was similar to that of pure drugs indicating the peak purity in all formulations. There was no significant interaction between the drugs and various excipients present in the pharmaceutical formulations (Table 7).

Table 7 – Analysis of simvastatin (SMV) and fenofibrate (FNF) in marketed products

Amount of drug in powder	FNF		SMV	
	% Recovery ± SD	RSD	% Recovery ± SD	RSD
5	99.93 ± 0.07	2.57	99.98 ± 0.06	2.30
25	99.90 ± 0.14	1.02	99.92 ± 0.15	0.96
50	100.03 ± 0.07	0.25	100.02 ± 0.08	0.29

SD = standard deviation.

3.5. *Application of developed method for the analysis of FNF and SMV in developed SEDDS*

The developed reversed phase-UHPLC method was found to be quick, sensitive enough, and suitable for the quantitative estimation of SMV and FNF. Therefore, this method was put to test for the estimation of SMV and FNF in the developed SEDDS. The amount of SMV and FNF in the SEDDS was found to be 100.45% and 100.17%, respectively. This high assay value and low %RSD (1.15% for SMV and 0.29% for FNF, respectively) of both drugs in developed SEDDS indicate that the method is suitable for simultaneous analysis of developed SEDDS in bulk drug and marketed products. The chromatogram of simultaneous analysis extracted from all the SEDDS formulations was matching with that of standard SMV and FNF, showing the purity of peak in tested formulations (Figure 3).

4. Conclusion

It can be concluded from the present study that methanol–water mixture can be employed as an environmentally

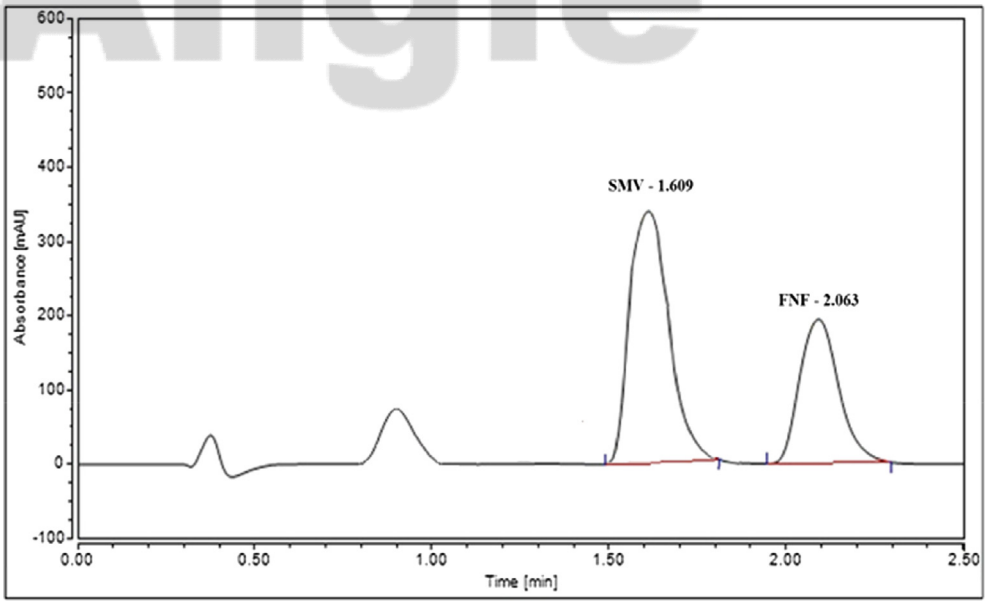

Figure 3 – Application of the developed *green* chromatographic method for the detection of simvastatin (SMV) and fenofibrate (FNF) in self-emulsifying drug delivery systems.

benign eluent for the simultaneous analysis of anti-hyperlipidemic drugs. Hydrophobic (nonpolar) interactions of the drugs with hydrophobic (or lipophilic) stationary phase C_{18} were the forces responsible for their separation. The developed method was found to be linear over a wide concentration range as well as exhibiting good precision, robustness, and recovery properties. The newly developed method was found applicable for the simultaneous separation of SMV and FNF in marketed formulations and SEDDS, indicating its suitability for the routine analysis of these drugs. Another important conclusion of this study was that these green eluents have the potential to be used for analysis in chromatographic science and thus making the separation process more environment friendly. Safer alternatives should be explored on the basis of their environmental, health, and safety and life-cycle assessment to replace traditional solvents making separation science green because developing green analytical methods to come in place of traditional ones is a crucial requirement.

Conflicts of interest

The authors report no conflict of interest related to this manuscript.

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REFERENCES

[1] Grundy SM, Gotto AM, Bierman EL. Recommendations for the treatment of hyperlipidemia in adults, A joint statement of the Nutrition Committee and the Council on Arteriosclerosis of the American Heart Association. Arteriosclerosis 1984;4:443A–68A.

[2] European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: a policy statement of the European Atherosclerosis Society. Eur Heart J 1987;8:77–88.

[3] European Atherosclerosis Society. The recognition and management of hyperlipidaemia in adults: A policy statement of the European Atherosclerosis Society. Eur Heart J 1988;9:571–600.

[4] Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci 2006;29:278–87.

[5] Vega GL, Ma PT, Cater NB, Filipchuk N, Meguro S, Garcia-Garcia AB, Grundy SM. Effects of adding fenofibrate (200 mg/day) to simvastatin (10 mg/day) in patients with combined hyperlipidemia and metabolic syndrome. Am J Cardiol 2003;91:956–60.

[6] Jones P. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin and fluvastatin in patients with hypercholesterolemia (The CURVES study). Am J Cardiol 1998;81:582–7.

[7] McCullough PA, Ahmed AB, Zughaib MT, Glanz ED, Di Loreto MJ. Treatment of hypertriglyceridemia with fibric acid derivatives: impact on lipid subfractions and translation into a reduction in cardiovascular events. Rev Cardiovasc Med 2011;12:173–85.

[8] Rohilla A, Dagar N, Rohilla S, Dahiya A, Kushnoor A. Hyperlipidemia—a deadly pathological condition. Int J Curr Pharm Res 2012;14:15–8.

[9] Schweitzer M, Tessier D, Vlahos WD, Leiter L, Collet JP, McQueen MJ, Harvey L, Alaupovic P. A comparison of pravastatin and gemfibrozil in the treatment of dyslipoproteinemia in patients with non-insulin-dependent diabetes mellitus. Atherosclerosis 2002;162:201–10.
[10] Leuner C, Dressman JB. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm 2000;50:47–60.
[11] Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self emulsifying and 'self-microemulsifying' drug delivery systems. Eur J Pharm Sci 2000;11:S93–8.
[12] Pouton CW. Formulation of self-emulsifying drug delivery systems. Adv Drug Del Rev 1997;25:47–58.
[13] Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm Res 1992;9:87–93.
[14] Patel AR, Vavia PR. Preparation and *in vivo* evaluation of SMEDDS (self-microemulsifying drug delivery system) containing fenofibrate. AAPS J 2007;9:E344–52.
[15] Bhavesh D, Shah S, Shivprakash. Determination of fenofibric acid in human plasma by ultra performance liquid chromatography–electrospray ionization mass spectrometry: application to a bioequivalence study. Biomed Chromatogr 2009;23:922–8.
[16] Skrha J, Stulc T, Hilgertová J, Weiserová H, Kvasnicka J, Ceska R. Effect of simvastatin and fenofibrate on endothelium in Type 2 diabetes. Eur J Pharmacol 2004;493:183–9.
[17] Wren SA, Tchelitcheff P. Use of ultra-performance liquid chromatography in pharmaceutical development. J Chromatogr A 2006;1119:140–6.
[18] Nagaraj VK, Rajshree M. Derivative ratio spectrophotometry and chemometric calibrations method is reported for simultaneous resolution of ATO and FEN. Anal Sci 2007;23:445–51.
[19] Kadav AA, Vora DN. Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets. J Pharm Biomed Anal 2008;48:120–6.
[20] Chou YC, Wang YK, Charng MJ, Ueng YF. Determination of serum atorvastatin concentrations in lipid-controlling patients with and without myalgia syndrome. J Food Drug Anal 2013;21:147–53.
[21] Anastas PT, Warner JC. Green chemistry: theory and practice. New York: Oxford University Press; 1998.
[22] Sheldon RA. Green solvents for sustainable organic synthesis: state of the art. Green Chem 2005;7:267–78.
[23] Mohammad A, Haq N, Siddiq A. Resolution of multicomponent mixture of amino acids using environmentally benign eluents: A green chromatographic approach. J Sep Sci 2010;33:3619–26.
[24] Mohammad A, Haq N. TLC separation of amino acids with a green mobile phase. J Planar Chromat Mod TLC 2010;23:260–4.
[25] Haq N, Iqbal M, Alanazi FK, Alsarra IA, Shakeel F. Applying green analytical chemistry for analysis of drug: adding health to pharmaceutical industry. Arab J Chem 2012. http://dx.doi.org/10.1016/j.arabjc.2012.12.004.
[26] Capello C, Fischer U, Hungerbuhler K. What is a green solvent? A comprehensive framework for the environmental assessment of solvents. Green Chem 2007;9:927–34.
[27] Koller G, Fischer U, Hungerbuhler K. Assessing safety, health, and environmental impact early during process development. Ind Eng Chem Res 2000;39:960–72.
[28] Environmental management. Life cycle assessment—Principles and framework. EN ISO 14040. Brussels: European Committee for Standardisation; 1997.
[29] Naushad MA, Aqil M, Ahmad FJ, Ali A, Faisal MS, Rizwan M, Faiyaz S. Development and validation of the HPLC method for the analysis of trimetazidine hydrochloride in bulk drug and pharmaceutical dosage forms. J Anal Chem 2008;63:965–70.
[30] Haq N, Shakeel F, Ali M, Elbadry M, Alanazi FK, Alsarra IA. An environmentally benign approach for rapid analysis of indomethacin using a stability indicating RP-HPLC method. J Liq Chromatogr Rel Technol 2014;37:878–92.