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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,