DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR GEMFIBROZIL AND SIMVASTATIN IN BINARY COMBINATION

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ABSTRACT

A simple, sensitive and validated HPLC method has been developed to determine gemfibrozil and simvastatin simultaneously in synthetic mixture form. Chromatographic separation was achieved on a C-18 column using a mixture of 0.1 M ammonium acetate pH 5.0 and acetonitrile in the ratio of 15:85 (v/v) at a wavelength of 237 nm. Linearity of the method was found to be in the concentration range of 60–420 µg/ml for gemfibrozil and 1–7 µg/ml for simvastatin with correlation coefficient greater than 0.9999. The total eluting time for the two components is less than ten minutes. The method can be used for simultaneous determination of gemfibrozil and simvastatin.

Key words: HPLC, acetonitrile, gemfibrozil, simvastatin

INTRODUCTION

Gemfibrozil (Fig. 1) chemically designated as 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid is a cholesterol-lowering agent. It has been found effective not only for reducing serum cholesterol, triglyceride, and LDL levels, but also effective for increasing serum HDL levels [1]. It has also been demonstrated that this drug lowers the incidence of coronary heart disease in humans [2-3]. A number of analytical methods have been developed for its determination in pharmaceutical formulations or in biofluids either alone or in combination with other drugs. These include determination of gemfibrozil by liquid chromatography–tandem mass spectrometry [4], HPLC [5-13], flow injection and spectrofluorimetric analysis [14-15].

Simvastatin (Fig. 2), a hypolipidemic drug belonging to the class of pharmaceuticals called statins is chemically designated as [(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate. It is used for the treatment of hypercholesterolemia [16]. Following conversion of this lactone prodrug to its hydroxyl acid form, the compound is a potent competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis [17]. Different analytical methods have been reported for the determination of simvastatin, which include HPLC [18-21], HPLC-MS/MS [22], derivative spectrophotometry [23] and Voltammetric techniques [24].

Fig.1. Chemical structure of Gemfibrozil

Fig.2. Chemical structure of simvastatin

Many patients with coronary artery disease do not respond well with single-agent therapy. The combination of an HMG-CoA reductase and gemfibrozil are ideal and recent reports confirm the efficacy of combinations of gemfibrozil and an HMG-CoA reductase [25-32].

Combination of statins with fibrates can be used for combined dyslipidemia and it can decrease low density lipoprotein cholesterol more than 40 % [33]. Controlled trials have not only shown regression of atherosclerotic lesions with this combination, but have also demonstrated increased risk of myopathy [34-35]. Experts believe myopathy risk is greater with gemfibrozil as compared to fenofibrate, [36]. The maximum approved daily doses of lovastatin, simvastatin and rosuvastatin are therefore lowered (20, 10 and 10 mg, respectively) when used with gemfibrozil [37].

According to the information collected from literature there is no reported method for simultaneous determination of gemfibrozil and simvastatin. In the present work we are therefore focused on to achieve the optimum chromatographic conditions for the simultaneous determination of gemfibrozil and simvastatin in a synthetic mixture.

We describe in this paper a simple, sensitive and validated HPLC method with total run time less than ten minutes for the simultaneous determination of gemfibrozil and simvastatin. The developed method can be applied successfully to quality control and for other analytical purposes.

EXPERIMENTAL

1. Chemicals and Reagents

Gemfibrozil and simvastatin reference substances with claimed purity of 99.70 % and 99.65 % respectively were taken from Atco Laboratories (Karachi, Pakistan) and Schazoo Laboratories (Lahore, Pakistan) respectively. Acetonitrile (HPLC grade), ammonium acetate and acetic acid glacial (analytical reagent grade) were purchased from Merck (Lahore, Pakistan). All exceptients used were of pharmaceutical grade. Water for injection was used throughout the experiment. Mobile phase was filtered using 0.45 µm cellulose acetate filters made by Millipore (USA) whereas, Whatmann filter papers No.41 (purchased from the local market) were used in the preparation of sample solution in section 6.

2. Apparatus and chromatographic conditions

HPLC apparatus consisting of Shimadzu LC-10A system equipped with a model LC-10AT pump, an SPD-10A variable wavelength detector (set at 237 nm), a CBM-10A interface module with class LC-10 HPLC software and a Rheodyne injection valve with a 20 µL loop was used for development and evaluation of this method. A Merck C 18 column (250*4.6 mm, i.d., 5 µm particle size) was selected. The mobile phase was composed of a mixture of 0.1 M ammonium acetate pH 5.0 ± 0.05 and acetonitrile in the ratio of 15: 85 (v/v). An external standard method was used and the flow rate was 1 ml/min⁻¹. The HPLC system was operated at room temperature 25 ± 2°C.

3. Preparation of standard solution

A Stock solution of gemfibrozil and simvastatin was prepared at about 6.0 mg/ml and 0.1 mg/ml respectively in mobile phase. The working standard solution 240 µg/ml for gemfibrozil and 4 µg/ml for simvastatin were prepared by diluting the stock solution with mobile phase

4. Linearity

Linearity of the proposed method was checked by analyzing seven solutions in the range of 60–420 µg/ml for gemfibrozil (60, 120, 180, 240, 300, 360, 420 µg/ml) and 1–7 µg/ml for simvastatin (1, 2, 3, 4, 5, 6, 7 µg/ml). Each level was made in triplicate.

5. Accuracy

Method accuracy was performed by adding known amounts of gemfibrozil

and simvastatin to the pre-analysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 50, 100 and 150 % of the nominal analytical concentration (240 µg/ml for gemfibrozil and 4 µg/ml for simvastatin). Each level was made in triplicate.

6. Selectivity

The selectivity of the proposed method was checked by making a synthetic mixture of both the analytes with commonly occurring excipients that are found in most tablet formulations and then comparing the chromatogram with the chromatogram of the reference standard. Synthetic mixture containing 600 mg gemfibrozil, 10 mg simvastatin and 20 mg each of starch, lactose, magnesium stearate and avicel which are present as excipients in the pharmaceutical formulation were accurately weighed and transferred into 100 ml volumetric flask. The mixture was shaken well with 70 ml mobile phase and then the volume was completed with mobile phase and filtered. 1 ml of this filtrate was transferred into 25 ml volumetric flask and mobile phase was then added to volume to obtain a final solution containing 240 µg/ml gemfibrozil and 4 µg/ml simvastatin.

7. Robustness

Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition and flow rate of the mobile phase and pH of the buffer solution. The chromatographic parameters of each analyte such as retention time, tailing factor, resolution and number of theoretical plates were measured at each changed conditions.

8. Precision

For evaluating the within-day precision, results of five replicate analysis of three different concentrations of samples were calculated on a single day. The between-day precision was calculated from the same samples analyzed on five different days.

9. LOD and LOQ

For calculating the LOD and LOQ values, solutions with known decreased concentrations of analytes were injected into the HPLC system. The limit of detection (LOD) and quantification (LOQ) were then measured by calculating the minimum level at which the analytes can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1) with accuracy, respectively.

RESULTS AND DISCUSSION

In the present work conditions were optimized for the development and validation of a simple and accurate HPLC method for the simultaneous determination of gemfibrozil and simvastatin in synthetic mixture form. Method development was started with 0.1 M ammonium acetate pH 5.0 and acetonitrile in the ratio of 30:70 (v/v) based on our previous results for atorvastatin and simvastatin (38-39). At this composition although both components were eluted but resolution was greater than 20 and retention time of simvastatin was about 16 minutes. The acetonitrile contents of the mobile phase were then increased to decrease resolution and retention time. At the composition of 15:85 (0.1 M ammonium acetate pH 5.0 and acetonitrile) both components were eluted with a good resolution.

The most appropriate mobile phase composition was thus found to be 0.1M ammonium acetate pH 5.0 and acetonitrile in the ratio of 15:85 (v/v). Under the described experimental conditions, sharp peaks that belong to gemfibrozil and simvastatin were obtained at retention times of 4.65 and 7.68 minutes respectively as shown in Figure.3.

Fig. 3. Chromatograms of Gemfibrozil and simvastatin reference substance

The developed chromatographic method was validated using ICH guidelines [40]. Validation parameters performed include linearity, limit of detection and quantitation, selectivity, robustness, accuracy and repeatability.

The calibration curve was linear over the concentration range of 60-420 µg/ml for gemfibrozil and 1-7 µg/ml for simvastatin. The correlation coefficient in both cases was found to be greater than 0.9999 which manifests a linear relationship between concentration and the peak area. The linear regression equation for gemfibrozil was found to be $Y = 5.112 X + 2.26$ with correlation coefficient equal to 0.99995. The linear regression equation for simvastatin was found to be $Y = 35.679 X - 0.365$ with value of correlation coefficient equal to 0.99997.

In this study, the LOD was found to be 0.13 µg/ml and 0.02 µg/ml for gemfibrozil and simvastatin respectively. The LOQ was found to be 0.39 µg/ml and 0.06 µg/ml for gemfibrozil and simvastatin respectively.

The recovery and the relative standard deviation for each of the analytes are given in Table.1.

Table.1. Accuracy of the proposed HPLC method.

Drug	level (%)	n	Added Conc. (µg/ml)	Found Conc. (µg/ml)	% recovery	% RSD
Gemfibrozil	50	5	120.0	120.85	100.71	0.86
	100	5	240.0	238.58	99.41	0.71
	150	5	360.0	356.51	99.03	0.42
Simvastatin	50	5	2.0	2.03	101.50	1.02
	100	5	4.0	4.08	102.00	1.45
	150	5	6.0	5.95	99.17	0.96

The results of within-day and between-day precision are presented in Table 2.

Table.2. Precision of the proposed HPLC method

Compound	Conc. (µg/ml)	n	Within-day precision		Between-day precision	
			Mean	RSD(%)	Mean	RSD(%)
Gemfibrozil	120.0	5	121.25	0.78	119.58	1.25
	240.0	5	244.56	0.95	242.58	1.02
	360.0	5	365.21	1.24	363.21	0.85
Simvastatin	2.0	5	2.02	1.44	2.01	1.06
	4.0	5	3.96	1.11	3.95	0.58
	6.0	5	6.07	0.36	6.02	1.31

The chromatogram of gemfibrozil and simvastatin in synthetic mixtures is given in figure 4 showing selectivity of the proposed method.

Fig. 4. Chromatograms of Gemfibrozil and simvastatin in a synthetic mixture

Robustness of the method was performed by intentionally modifying the chromatographic conditions. The results showed that the variance of the conditions had no appreciable effects to that of actual. The results of the robustness study are given in Table 4a & 4b.

Table 4a: Robustness study of Gemfibrozil

Conditions	t _R (min)	Theoretical plate	Tailing
Acetonitrile: buffer (85:15)	4.66	7823	1.23
Acetonitrile: buffer (80:20)	5.17	8214	1.20
Acetonitrile: buffer (90:10)	4.41	6310	1.25
Flow rate (1.1 ml/min)	4.24	7118	1.28
Flow rate (0.9 ml/min)	5.18	8002	1.22
Buffer (pH 5.2)	4.65	7719	1.23
Buffer (pH 4.8)	4.65	7662	1.23

Table 4b: Robustness study of Simvastatin

Conditions	t _R (min)	Theoretical plate	Tailing	Resolution
Acetonitrile: buffer (85:15)	7.68	11243	1.18	12.09
Acetonitrile: buffer (80:20)	8.19	11920	1.15	13.56
Acetonitrile: buffer (90:10)	6.45	9215	1.25	8.19
Flow rate (1.1 ml/min)	6.98	10220	1.29	10.59
Flow rate (0.9 ml/min)	8.53	12515	1.22	14.55
Buffer (pH 5.2)	7.65	11308	1.22	11.95
Buffer (pH 4.8)	7.66	11015	1.22	11.95

CONCLUSION

A simple and accurate reverse phase HPLC method has been developed for the simultaneous determination of gemfibrozil and simvastatin. The method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation, selectivity and robustness. The run time of less than ten minutes allows its application for the routine determination of gemfibrozil and simvastatin.

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