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A Validated Reversed Phase Liquid Chromatographic Method for Simultaneous Determination of Some Antihyperlipidemic Drugs.

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ABSTRACT

A simple, sensitive, precise, fast and accurate reversed phase liquid chromatographic method has been developed for the simultaneous estimation of six antihyperlipidemic drugs. namely, rosuvastatin, bezafibrate, ezetimibe, atorvastatin, fenofibrate and simvastatin. The method was developed using a reversed-phase C_{18} column with a mobile phase consisting of water adjusted to pH 4.4 by orthophosphoric acid : acetonitrile (30 : 70 v/v) at a flow rate of 1 mL/min. and ultraviolet detection at 242 nm. The retention times ranged from 3.23 to 20.13 min. Under the optimum chromatographic conditions correlation coefficients were in the range of 0.9990 – 0.9997, linearity was from 0.06 to 20 µg/mL with detection limits from 0.02 to 0.10 µg/mL and mean recoveries of 95.67 ± 1.15 to 102.78 ± 1.86 %. The developed method was validated according to ICH guidelines and could be applied for the estimation of the studied drugs in their pure and combined dosage forms.

Keywords: HPLC ; Antihyperlipidemic drugs; Pharmaceutical analysis.

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INTRODUCTION

Cholesterol plays a crucial role in maintaining cell membrane integrity and physiological functions of the body, including steroid hormone synthesis. However, high levels of cholesterol are associated with serious pathological conditions such as atherosclerosis which is characterized by deposition of cholesterol in the arterial wall. Atherosclerosis of the coronary and peripheral vasculature is the leading cause of death worldwide. Lowering cholesterol levels can arrest or reverse atherosclerosis in all vascular beds and significantly decrease the morbidity and mortality associated with atherosclerosis. Each 10 % reduction in cholesterol levels is associated with ~20 – 30% reduction in the incidence of the coronary heart diseases [1]. Lipid regulating drugs are used to modify blood lipid concentrations in the management of hyperlipidaemias and for the reduction of cardiovascular risk. The principal groups of lipid regulating drugs are the statins, fibrates, bile-acid binding resins, nicotinates, omega-3 triglycerides and ezetimibe [2]. The most common groups of antihyperlipidemic medicaments are; statins, which act by inhibiting the rate limiting key enzyme in cholesterol biosynthesis [3] such as atorvastatin (ATOR), rosuvastatin (ROS) and simvastatin (SIM) The second group is fibrates which are highly effective agents for the treatment of atherogenic dyslipidemias [4] as fenofibrate (FENO) and bezafibrate (BEZA). Ezetimibe (EZE) is the sole member of the third group which is a selective cholesterol absorption inhibitor that inhibits the absorption of biliary and dietary cholesterol from the small intestine without affecting the absorption of fat soluble vitamins, triglyceride or bile acids [5]. In severe hyperlipidemic cases, synergistic treatment by different groups of antihyperlipidemics either co-administered or in combined dosage forms is required. Some chromatographic methods were developed for the determination of these drugs alone [6 – 8], binary [9 – 14] or ternary [15] mixtures with other hypolipidemics, and with other cardiovascular drugs [16 – 18]. Three statins were determined densitometrically at different wavelengths with low sensitivity [19]. A number of statins were simultaneously analyzed by either isocratic (four statins and each pair isocratically with a different eluent) [20] and gradient [1, 21] elution HPLC systems. Capillary electrophoresis (CE) was used efficiently for the simultaneous determination of fibrates [22] and statins [23], All the previously mentioned chromatographic methods could be applied for the simultaneous analysis of some antihyperlipidemics of the same group, and thus could not be applied for the simultaneous analysis of combined or co-administered lipid regulating drugs. To-date, there is no published report for the simultaneous isocratic analysis of six antihyperlipidemics from three different groups which are likely to be combined together in severe hyperlipidemic cases. The aim of the present work is to develop and validate an HPLC method for the simultaneous determination of six antihyperlipidemic drugs in bulk and formulations.

The studied drugs are, ROS; bis[(E)-7[4-(4- fl uorophenyl)-6-isopropyl-2-[methyl (methyl-sulphonyl) amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt [24], BEZA; 2 - (4-{2-[(4-chlorobenzoyl) amino] ethyl} phenoxy) - 2 - methylpropanoic acid [25], EZE; [1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone] [26], ATOR; [R-(R*,R*)] - 2 - (4 - fluorophenyl) - β, δ – dihydroxy – 5 - (1-methylethyl) – 3 – phenyl – 4 -[(phenylamino)carbonyl]- 1H- pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, FENO; propan-2-yl 2-{4-[(4-chlorophenyl)carbonyl] phenoxy}-2-methylpropanoate [27] and SIM;. (1S, 2S, 8S, 8aR)-1,2,

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6,8,8a-hexahydro-1-(2-((2R,4R)-terahyro-4-hydroxy-6-oxo–2H-pyran-2-yl)-2,6-dimethyl napthalen- 8-yl)2,2- dimethylbutanoate [28]. The chemical structures of the investigated drugs are illustrated in the following Fig. (1):

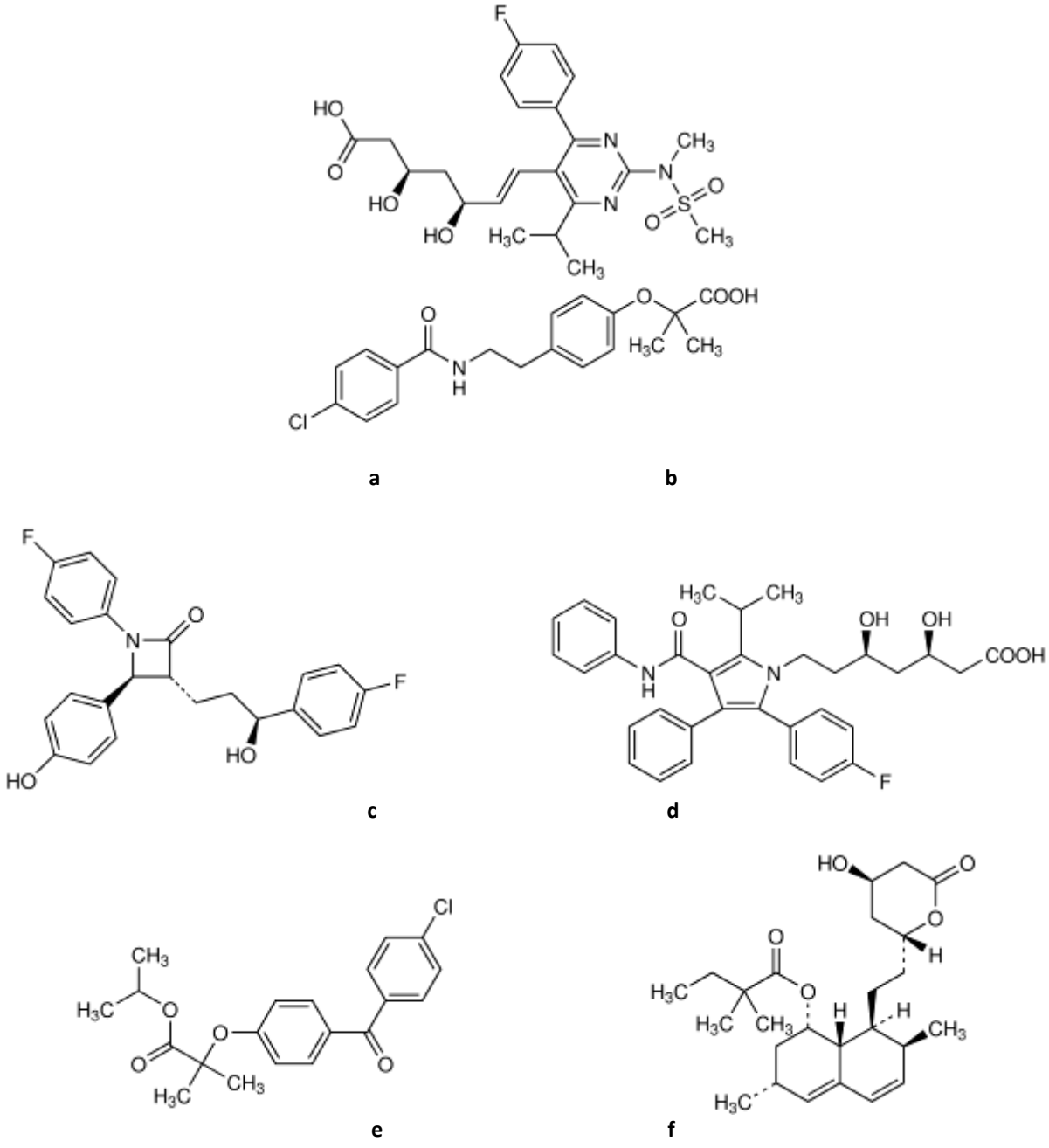

Fig. (1)

MATERIALS AND METHODS

Pharmaceutical grade ROS was purchased from Astrazeneca Egypt, New Maadi, Cairo, Egypt. Atorvastatin and BEZA were kindly supplied by EIPICO pharmaceuticals Co., 10th of Ramadan city, Cairo, Egypt as a gift. Ezetimibe was purchased from Alhikma Pharma S.A.E., 6 October city, Egypt. Simvastatin was kindly provided by Pharco Pharmaceuticals Co., Alexandria, Egypt. Fenofibrate was kindly sourced by Minapharm pharmaceuticals and

chemical industries Co., 10th of Ramadan city, Cairo, Egypt. Methanol and acetonitrile from Sigma-Aldrich, St. louis, USA. All solvents and chemicals were of analytical grade. Pharmaceutical formulations containing the studied drugs were obtained from the local market. Lipitor 10® tablets (10 mg ATOR) (Pfizer Egypt, Almaza, Cairo, Egypt), Choletimb 10® tablets (10 mg EZE), Zocor 10® tablets (10 mg SIM) (Global Napi Pharmaceuticals Co., 6th October city, Egypt), Lipanthyl supra® tablets (160 mg FENO) (Minapharm pharmaceuticals Co.,10th of Ramadan city, Egypt), Crestor 20® (20 mg ROS) (Astrazeneca Egypt, New Maadi, Cairo, Egypt) in addition to Bezalip Retard® tablets (200 mg BEZA) which were laboratory prepared.

Preparation of standard solutions

Stock solutions containing 100µg/mL of all the investigated drugs were prepared in methanol. Working standard solutions were prepared by further dilution of suitable volumes of stock solutions with methanol to obtain concentrations in the range of 0.06 – 20 µg/mL for all the studied drugs.

Mixed drug solutions

Into 10 mL volumetric flasks, known accurate volumes of stock standard solutions of the studied drugs were mixed. Mixtures were completed to the mark with methanol then subjected to further dilutions to get final concentrations within their quantitation limits according to the proposed method.

Apparatus and Chromatographic Conditions

A Younglin Autochro-3000 HPLC system (Younglin, Korea) with UV detector, Rheodyne injection valve with 20-µL loop was used. Kromasil 100 C_{18} reversed-phase column of 250mm×4mm i.d., 5µm dimensions (VDS Optilab, Chromatographie technik GmbH, Germany). The mobile phase was a mixture of water adjusted to pH 4.4 by orthophosphoric acid and acetonitrile (30 : 70, v/v) and filtered through 0.45 µm pore size membrane filter of 30 mm diameter (Gelman® instrument co.). The flow rate was 1.0 mL/min and detection was carried out at 242 nm. The mobile phase was filtered and degassed by sonication before use. Peak identity was confirmed by comparison of the spectra and retention times with those of their respective standards.

Validation of the method

The proposed method was validated according to International Conference on Harmonization (ICH) guidelines [29].

Linearity

Calibration curves were constructed by analyzing a series of diluted stock solutions of the investigated drugs. Each solution was prepared in triplicate. Peak areas were plotted versus their respective concentrations and linear regression analysis were performed on the resultant curves. Linearity of the investigated drugs were studied over the following

concentration ranges; 0.33 – 5, 0.16 – 1, 0.1 – 1, 0.16 – 3, 0.06 – 5 and 0.2 – 20 µg/mL for ROS, BEZA, EZE, ATOR, FENO and SIM respectively.

Limits of detection and quantification

The LOD was determined based on either signal-to-noise ratios or using an analytical response of three times of the background noise. The LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections [30].

Accuracy and precision

Accuracy was evaluated as percentage relative error between the found mean concentrations and added concentrations of the authentic solutions [31], Accuracy was assessed by determination of the recovery of the method at three different concentration levels (corresponding to 80, 100, and 120% of the test solutions concentrations) by the addition of known amounts of the standards to the control preparations. For each concentration, three sets were prepared and injected. The intra- and inter-day variability or precision data were assessed by using standard solutions prepared to produce solutions of three different concentrations within the calibration range of each drug. Repeatability or intra-day precision was investigated by injecting three replicate samples of each of the samples of three different concentrations. Inter-day precision were assessed by injecting the same three samples over three consecutive days [30].

Robustness

The robustness of an analytical method is an estimation of its capability to remain unaffected by small but deliberate changes in method variables. It was determined by measuring % CV of peak areas ratios of the studied drugs before and after small variations in the pH and acetonitrile percent of the mobile phase.

System suitability

In order to determine the adequate resolution and repeatability of the proposed method, suitability parameters including retention factor, selectivity, resolution and asymmetry factor were investigated.

Analysis of the dosage forms

Accurately weighed amounts of powders obtained from 10 tablets equivalent to 10 mg of each drug were transferred separately into 10 mL volumetric flasks. About 5 mL methanol was added and the flasks were sonicated for 15 min, and then completed to 10 mL with methanol and mixed. The solutions were filtered and first portions of filtrates were rejected. Into 10 mL volumetric flasks, certain volumes of each prepared solution were

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transferred and then mixed well to give mixtures of the investigated drugs containing either EZE or BEZA. Aliquots of 20-µL were injected.

RESULTS

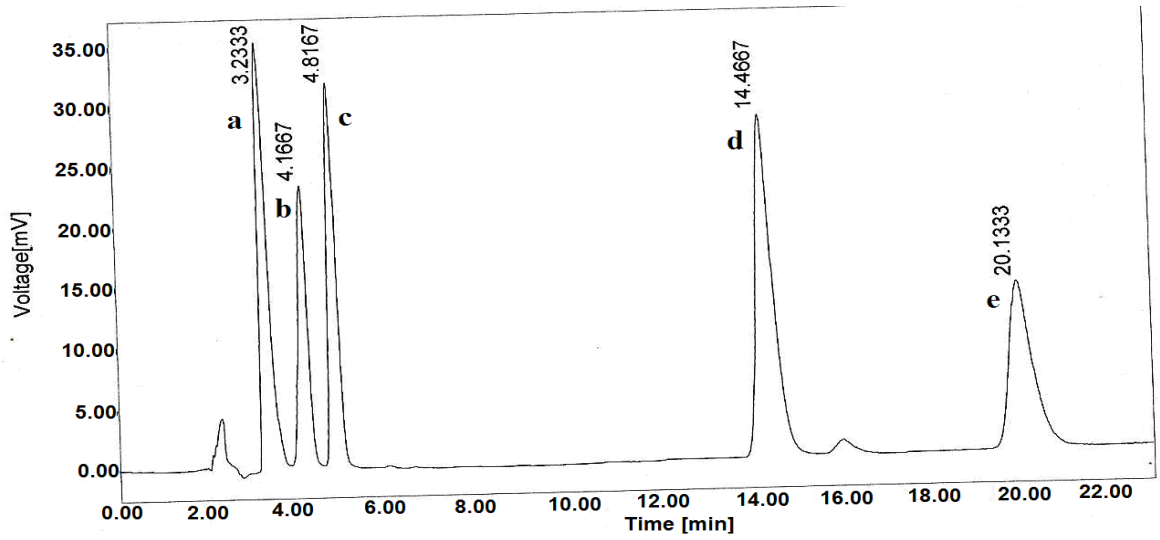
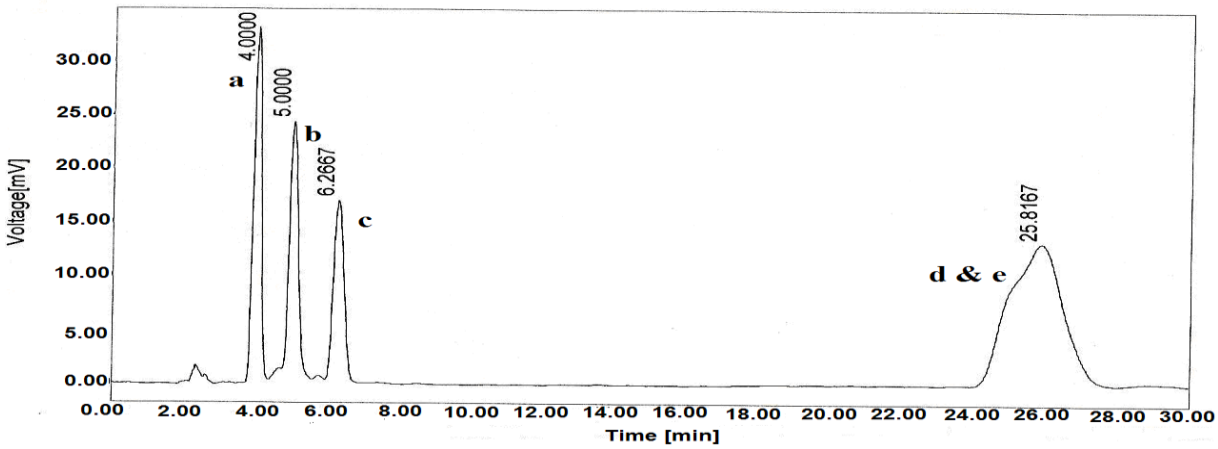
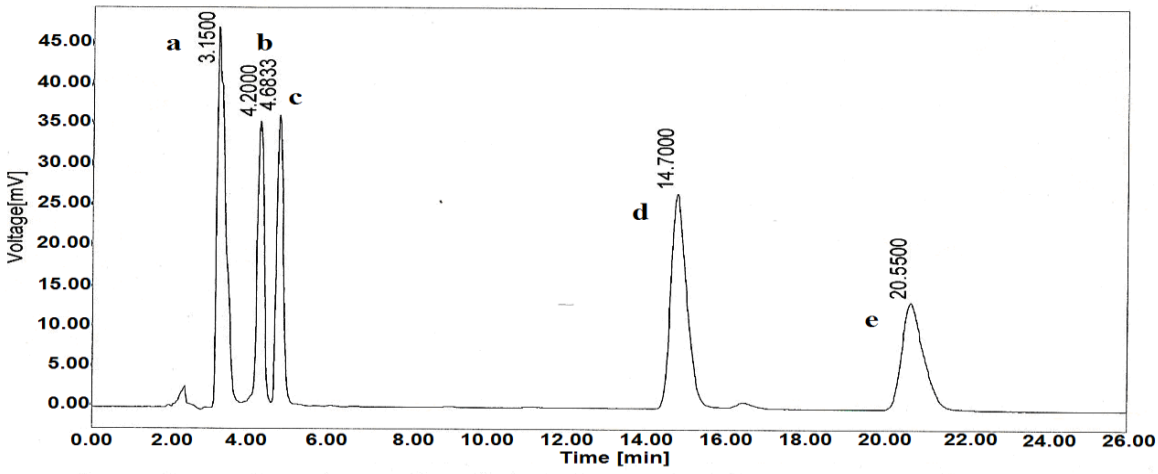


Fig. (2)



(a)



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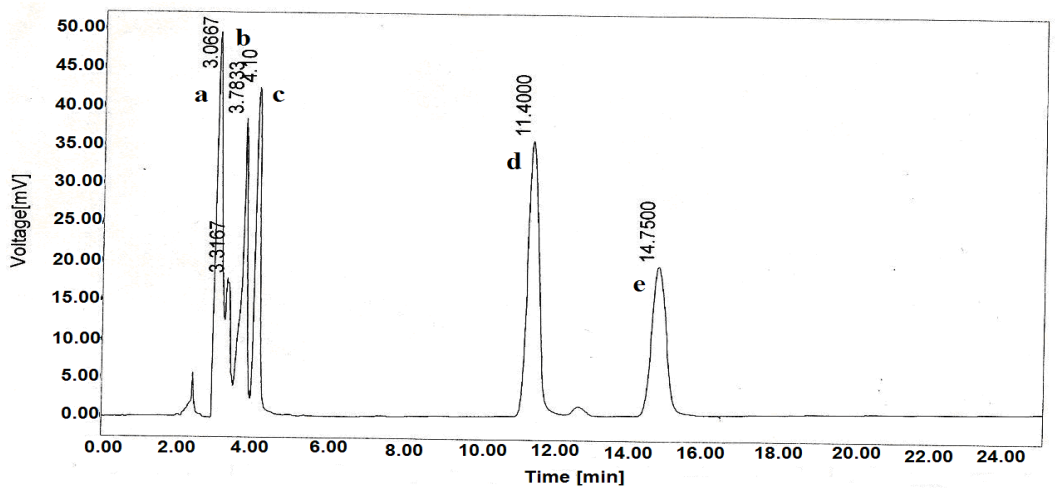
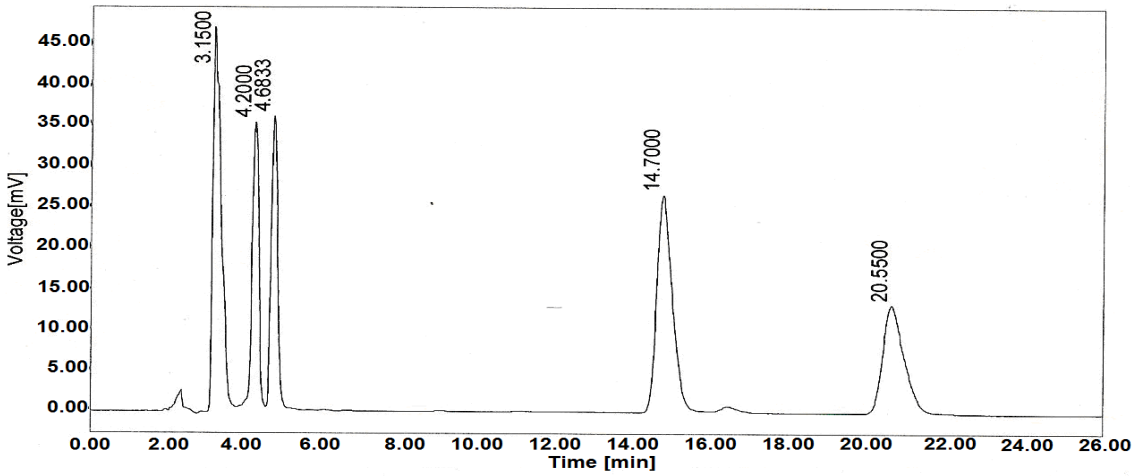


(c)

Fig. (3)



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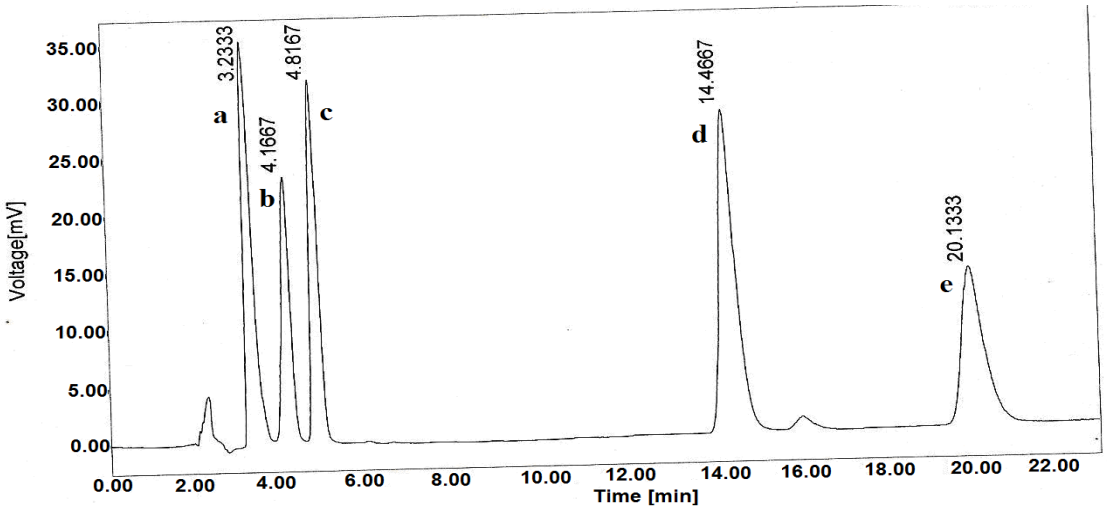

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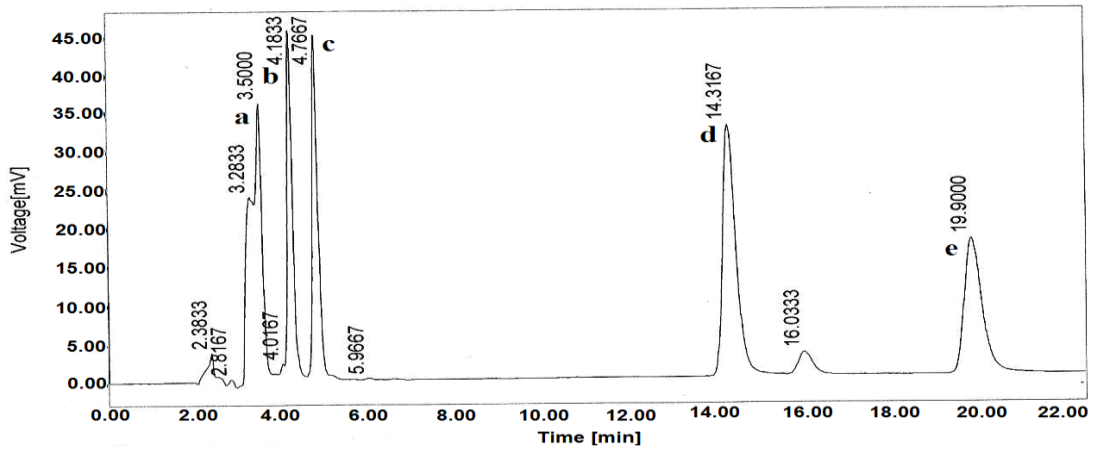
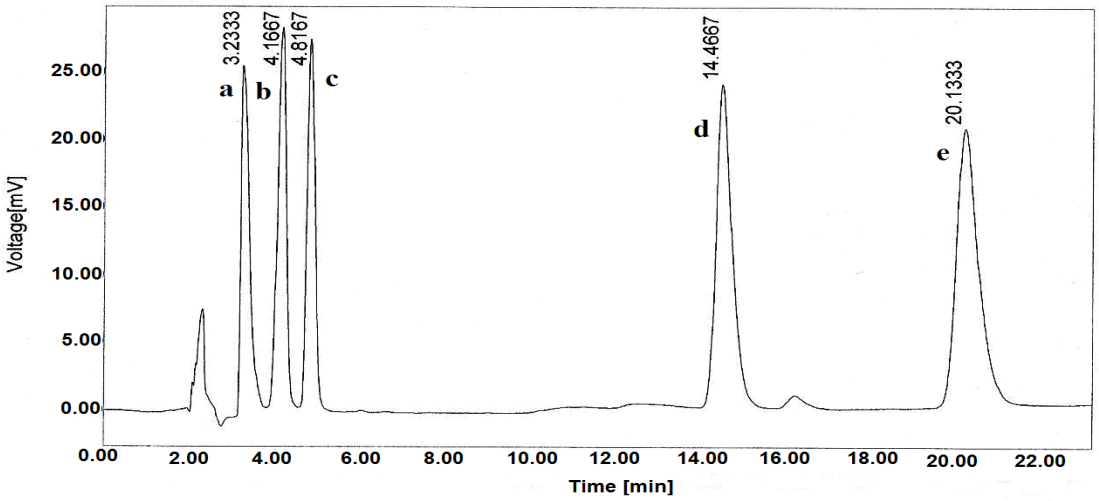


(c)

Fig. (4)



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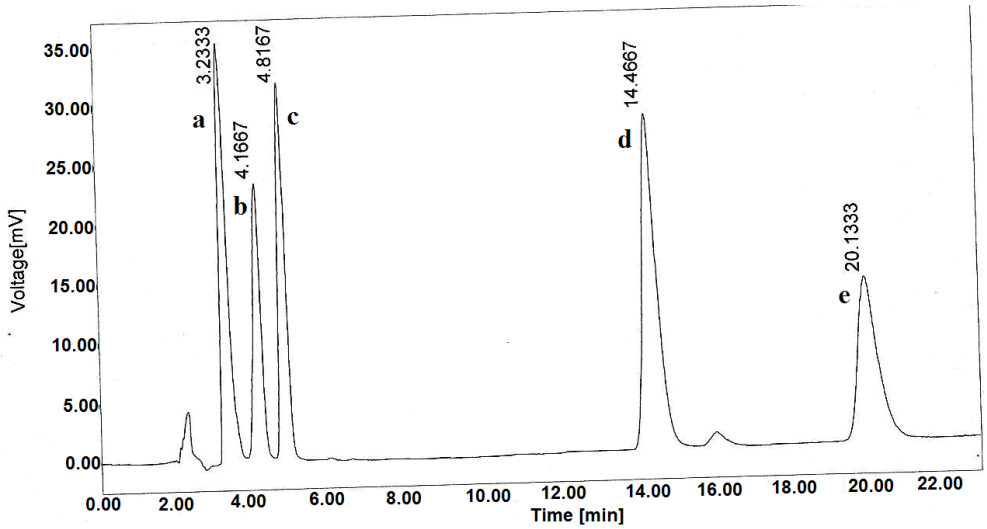

(b)

Fig. (5)

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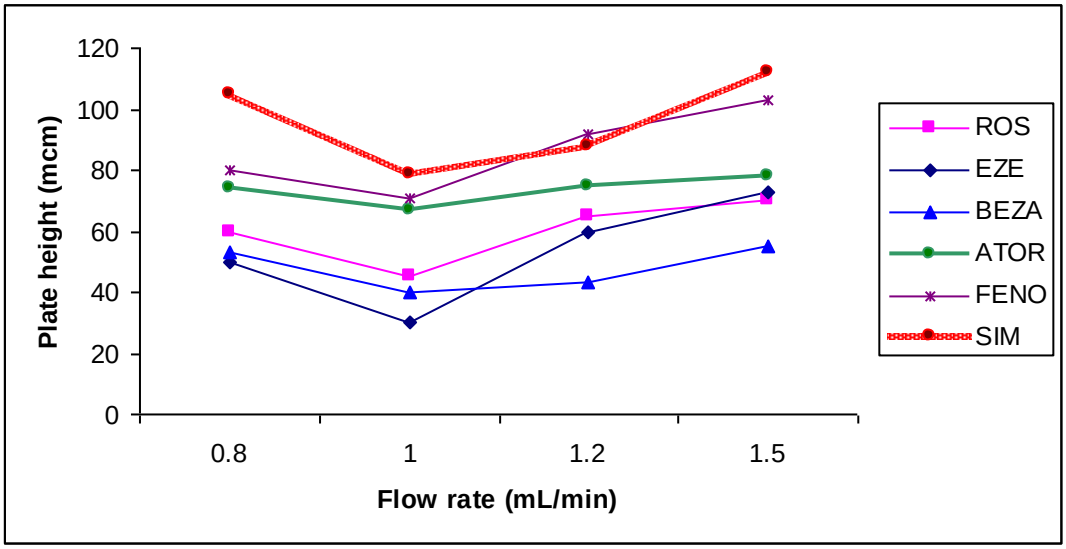

Fig. (6)

Method development

Optimization of the chromatographic conditions

Mobile phase composition

Mobile phases of different composition containing either acetate or phosphate buffers with acetonitrile have been tried. When buffers were replaced by water which was adjusted to acidic pH with orthophosphoric acid, better peak shapes and stable baseline were obtained. Upon testing different organic phase (methanol and acetonitrile) together with water at different ratios, best chromatographic conditions were attained with 70 % acetonitrile without methanol. Fig. (2)

The effect of the pH of the mobile phase

The eluent pH affects the ionization of inorganic species and consequently their retention. Different pH values for water adjusted with orthophosphoric acid from pH 3.0 – 5.2 were tried and best results were gotten with pH 4.4.

Detection wavelength

The studied drugs were detected at different wavelengths; 220, 230, 541 and 254 nm to determine the most proper one. Among the investigated wavelengths, the best results were obtained at 230 and 242 nm, but better sensitivities were depicted at 242, so it was selected as the wavelength of choice for our developed chromatographic procedure. Fig. (5)

Flow rate

Different flow rates (0.8 – 1.5 mL/min) were investigated to attain good resolution for the studied drugs with the least analysis time, and the most proper results obtained with 1 mL/min.

Analytical method validation

Linearity

Assay linearity was evaluated by the calibration graphs Excellent linear relationships between peak areas and concentrations were exhibited for the investigated drugs with highly significant correlation coefficients ranging from 0.9989 to 0.9997. Table (1)

Limits of detection and quantification

The least limit of detection (LOD) and quantitation (LOQ) for all the studied drugs were 0.02 and 0.06 respectively depicting the high sensitivity of the proposed method. Table (1) presents statistical parameters of the proposed method.

Accuracy and precision

Average percent recoveries for ROS, BEZA, EZE, ATOR, FENO and SIM were; 96.0, 98.8, 97.3, 99.8, 97.7 and 100.7 % respectively, while % CV values were < 2 % indicating accuracy of the reported method. Table (2) presents the accuracy of the proposed method. Precision data which are representing intermediate precision (intra-day and inter-days reproducibility) are summarized in Table (3). The % CV values for both intra-day and inter-days were ≤ 2 %, which indicates that the proposed method is reliable and precise.

Robustness

Minor deliberate changes in different experimental parameters; acetonitrile percent, mobile phase pH and detection wavelength were found not to significantly affect the recoveries, peak areas and retention times of the investigated drugs. The coefficient of variation values were not exceeding 2.5 % indicating that the proposed method is robust. Table (4).

System suitability

Excellent suitability parameters were obtained and the results were abridged in Table (5).

Analysis of the dosage forms

Some commercial dosage forms of the studied drugs were successfully analyzed by the developed HPLC method and recovery experiments were carried out for the studied drugs in their respective pharmaceutical formulations. The recoveries ranged from 97.7 ± 1.7 to 103.7 ± 1.4 % Table (6), depicting that the extraction method is convenient for all the

investigated drugs with good recoveries and there is no interference from the common excipients.

Table (I) Summary for the quantitative parameters and statistical data using the proposed method

Drug	Intercept (a) ± SD*	Slope (b) ± SD*	Linearity range (µg/ml)	Correlation coefficient (r)	Determination coefficient (r^2)	LOD (µg/ml)	LOQ (µg/ml)
ROS	0.9 ± 1.6	49.9 ± 1.2	0.3 – 5.0	0.9990	0.9982	0.11	0.33
BEZA	0.8 ± 1.1	71.5 ± 0.7	0.2 – 1.0	0.9995	0.9992	0.05	0.16
EZE	1.8 ± 0.6	68.0 ± 15	0.1 – 1.0	0.9997	0.9995	0.03	0.10
ATOR	2.5 ± 1.0	62.4 ± 0.3	0.2 – 3.0	0.9994	0.9996	0.05	0.16
FENO	-4.3 ± 0.6	445.0 ± 2.0	0.1 – 5.0	0.9997	0.9996	0.02	0.06
SIM	-30.2 ± 1.2	39.5 ± 2.0	0.2 – 20.0	0.9992	0.9985	0.06	0.20

* Average of three determinations

Table (II) Accuracy of the proposed method

Mixture components	The three concentration levels (µg/mL)		
	Standard added amount (µg/mL)		
	Recovery (%) ± SD[a]		
	CV[b] %		
ROS	3.6	4.0	4.4
	1.6	2.0	2.4
	93.7 ± 1.8	98.8 ± 1.5	95.2 ± 1.2
	1.9	1.5	1.3
BEZA	0.7	0.8	0.9
	0.3	0.4	0.5
	97.8 ± 1.2	102.5 ± 1.1	96.3 ± 0.6
	1.2	1.1	0.6
EZE	0.7	0.8	0.9
	0.3	0.4	0.5
	99.2 ± 1.6	94.7 ± 0.8	97.7 ± 0.6
	1.6	0.9	0.6
ATOR	2.2	2.4	2.6
	0.9	1.2	1.4
	98.7 ± 1.4	100.5 ± 1.4	100.3 ± 1.6
	1.4	1.3	1.6
FENO	3.6	4.0	4.4
	1.6	2.0	2.4
	96.1 ± 0.3	98.2 ± 1.5	98.8 ± 0.6
	0.4	1.5	0.6
SIM	14.4	16.0	17.6
	6.4	8.0	9.6
	102.8 ± 1.5	97.5 ± 0.3	101.8 ± 0.6
	1.5	0.3	0.6

[a] Average of three determinations.
[b] Coefficient of variation.

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Table (III) Intra- and inter-day precision of the proposed method

Mixture components	Concentration (µg/mL)	Intra-day precision		Inter-day precision	
		Recovery % ± SD[a]	% CV[b]	Recovery % ± SD[a]	% CV[b]
ROS	1.0	96.0 ± 1.4	1.5	98.5 ± 0.5	0.6
	2.5	100.3 ± 0.4	0.4	98.8 ± 1.2	1.2
	4.0	99.1 ± 1.5	1.5	101.7 ± 0.7	0.7
BEZA	0.2	102.8 ± 0.8	0.7	99.1 ± 0.7	0.7
	0.5	95.2 ± 0.2	0.2	97.7 ± 0.9	0.9
	0.8	97.4 ± 1.8	1.9	98.5 ± 0.1	0.1
EZE	0.2	98.3 ± 1.6	1.6	101.0 ± 0.8	0.8
	0.5	98.8 ± 0.5	0.5	97.5 ± 1.3	1.4
	0.8	98.8 ± 1.2	1.2	98.9 ± 1.9	1.9
ATOR	1.0	102.2 ± 1.7	1.7	95.0 ± 1.2	1.3
	1.5	99.5 ± 1.6	1.6	98.8 ± 0.9	0.9
	2.5	100.7 ± 0.8	0.8	101.1 ± 1.4	1.3
FENO	1.0	98.0 ± 1.1	1.1	96.6 ± 1.0	1.1
	2.5	95.3 ± 1.5	1.6	100.5 ± 2.0	2.0
	4.0	102.4 ± 1.3	1.3	97.6 ± 1.3	1.4
SIM	5.0	99.2 ± 1.5	1.6	98.5 ± 2.0	2.0
	10.0	101.5 ± 0.8	0.8	102.3 ± 1.7	1.7
	15.0	96.1 ± 1.3	1.3	102.0 ± 1.5	1.5

[a] Average of three determinations.
[b] Coefficient of variation.

Table (IV) Robustness of the proposed method

Mixture components / **Variables**	ROS		BEZA		EZE		ATOR		FENO		SIM	
	% Recovery ± SD	%CV[c]	% Recovery ± SD	%CV[c]	% Recovery ± SD	%CV[c]	% Recovery ± SD	%CV[c]	% Recovery ± SD	%CV[c]	% Recovery ± SD	%CV[c]
No variation[b]	98.4 ± 1.2	1.3	99.6 ± 0.4	0.4	101.0 ± 1.9	1.9	101.6 ± 0.1	0.1	98.0 ± 1.9	1.9	98.5 ± 0.8	0.8
Acetonitrile percent (± 0.5)	96.3 ± 0.4	0.5	102.2 ± 1.1	1.1	95.6 ± 0.5	0.5	98.6 ± 1.2	1.3	100.2 ± 0.8	0.8	103.3 ± 1.5	1.4
Mobile phase pH (± 0.2)	101.7 ± 1.6	1.6	97.0 ± 1.3	1.4	100.2 ± 1.5	1.5	99.2 ± 1.6	1.6	99.0 ± 1.2	1.2	99.1 ± 0.6	0.6
Detection wavelength (± 5.0 nm)	99.1 ± 0.6	0.6	97.8 ± 1.8	1.8	98.8 ± 2.3	2.3	98.5 ± 0.9	0.9	97.6 ± 1.7	1.7	99.4 ± 1.3	1.4

[a] Average of three determinations of peak area.
[b] Following the general assay procedure conditions.
[c] Coefficient of variation.

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Table (V) System suitability test parameters for the investigated drugs by the proposed method

System suitability test parameters	ROS	BEZA	EZE	ATOR	FENO	SIM
Retention time (min) (mean ± SD, n = 3)	3.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.7 ± 0.1	14.7 ± 0.1	20.5 ± 0.1
Repeatability of retention time; RSD[a] % (n = 3)	2.2	1.4	1.9	2.3	0.5	0.4
Tailing factor (asymmetric factor)[b]	1.0	1.0	1.0	1.1	1.2	1.4
Retention factor (k')[c]	0.4	1.0	1.0	1.1	5.7	8.3
	ROS – BEZA	ROS – EZE	BEZA – ATOR	EZE – ATOR	ATOR – FENO	FENO – SIM
Resolution (R_s)[d]	3.0	3.0	1.6	1.6	22.3	8.1
Selectivity factor (α)[e]	0.2	0.2	1.1	1.1	5.2	1.4

[a] RSD % = (SD / mean) × 100
[b] Calculated at 5 % peak height.
[c] $k' = (t_r - t_0) / t_0$, where t_r is the retention of analytes and t_0 is the column dead-time.
[e] $R_s = 2 (t_2 - t_1) / (w_2 + w_1)$. Where t_2 and t_1 are the retention of the second and first peaks w_2 and w_1 are the peaks widths of the second and first peaks.
[d] Separation factor, calculated as k_2 / k_1

Table (VI): Determination of the studied drugs in tablets using the proposed method.

Dosage form	Drug content (mg)	% Recovery ± SD[a]	Found amount (mg)	% CV[b]
Crestor tab.®	ROS (20.0)	103.7 ± 1.4	20.7	1.3
Bezalip tab.®	BEZA (100.0) (lab. synthized)	101.2 ± 1.4	101.2	1.4
Zetajon tab.®	EZE (10.0)	98.3 ± 0.6	9.8	0.6
Ator tab.®	ATOR (10.0)	101.3 ± 0.8	10.1	0.8
Lipanthyl supra tab.®	FENO (160.0)	97.7 ± 1.7	156.3	1.7
Zocor tab.®	SIM (10.0)	99.1 ± 1.2	9.9	1.2

[a] Average of three determinations.
[b] Coefficient of variation.

DISCUSSION

Mobile phase composition

Phosphate and acetate buffer trials resulted in poor peak shapes, unstable baselines, sharp increase in pressure and pump blockade due to precipitation of buffers'salts upon mixing with the organic phase.

Different solvent systems, including water adjusted to pH 3.0 mixed whether with methanol, acetonitrile or both at different ratios were pumped to optimize the mobile phase proportions, reduce retention times and enable good resolution with excellent peak

shapes for the studied antihyperlipidemics. Chromatographic trials have illustrated that by decreasing methanol percent with increasing acetonitrile percent by the same proportion at a constant aqueous phase percent would improve resolution, sensitivity and decrease retention times, for all the analytes. Methanol has led to peak broadening as a result of high viscosity and thus creating excessive high backpressure in the column. Meanwhile, inclusion of methanol molecules in the analyte solvated shell has added some hydrophobicity to the solvated molecular cluster, and this might have led to a significant distortion of the peak similarities and hence resolution of the investigated drugs [32].

The peak splitting of the acidic ROS (pKa 4.64) by further elevation of acetonitrile content could be due to the fact that the pKa values of acids increase with the addition of the organic modifier [33], and hence resulted in slightly directing the established equilibrium to more ionized forms rendering the percent of ionized forms below 90 % and leading to peak splitting. The HPLC retention could be explained as the result of competitive interactions of the analyte and eluent molecules with the stationary phase. Acetonitrile has high eluent strength since it forms a thick multi-molecular adsorbed layer on the surface of the reversed-phase adsorbent. From this point of view, the stronger the eluent interactions with the adsorbent surface, the lower the analyte retention [32] and hence the more the acetonitrile content the less the retention of the analytes. This interprets the overlapping between BEZA or EZE with ROS and ATOR peaks upon increasing acetonitrile percent to 75. Fig. (3) shows resolution improvement at different mobile phase ratios.

The effect of the pH of the mobile phase

Any compound in its ionic form is more hydrophilic, so it tends to have less interaction with hydrophobic stationary phases to be more solvated with protic solvents and hence less retention [32]. The more the ionization, the less retention of the analytes. It could be noted that fully protonated ROS (pKa = 4.6) in its aminopyrimidine moiety at the highly acidic medium gave rise to a strong cationic form. This form has showed the lowest retention among all the analytes since it became the most hydrophilic form and its interactions with the hydrophobic stationary phases were reduced. Similarly, the secondary amino group of the zweitter ion of BEZA (pKa = 3.37) and tertiary one in the 2-azetidinone ring in EZE (pKa = 9.75) were also protonated at this acidic pH and thus their interactions with the hydrophobic stationary phases were suppressed by the same value but to a lower extent than that of ROS, which accounted for their retarded elution. Although ATOR (pKa = 4.3) possesses secondary amino group and another tertiary one in the pyrrole ring, the possibility of its protonation is less because of the great chance of steric hindrance in such large molecular entity (Fig. 1d) and hence it has shown weaker repulsion with the unionized stationary phases. The studied pH range (3 – 5.2) minimized interconversion of SIM lactone into its β-hydroxy acid form [34] and consequently decreased the chance of the presence of ionized forms of its β-hydroxy acid and ensuring their stronger hydrophobicity.

The greater tendency of SIM β-hydroxy acid form to the lipophilic stationary phase resulted in its elution after FENO which is a highly lipophilic molecule but with smaller molecular weight resulting and less tendency towards the lipophilic stationary phase and consequently eluted prior to the larger SIM molecule.

The chromatographic behavior of the investigated drugs at the acidic range (pH 3 – 5.2), shows best results at both pH 3 and 4.4 in terms of peaks' symmetries and resolution. Better sensitivities for the majority of the studied drugs were attained at pH 3, due to the possibility of protonation to produce quaternary amino groups and hence predomination of the ionized species resulting in sharper and sensitive chromatograms except for the lipophilic FENO and SIM molecules which were not affected by pH change. Although higher sensitivity at pH 3.0, ROS shows a slight shoulder compared to the symmetric ideal chromatogram at pH 4.4. A poor resolution was observed between BEZA or EZE and ATOR at pH 3.0 (R_s = 1.74) than that at pH 4.4 (R_s = 2.36) which directed our current study to select pH 4.4 as the optimum pH value although better sensitivity was obtained at pH 3.0. Fig. (4)

Flow rate

The effectiveness of separation (resolution, R_s) in HPLC analysis, is dependent on thermodynamic factors (retention and selectivity) and kinetic factors (peak width and column efficiency) [35]. The relationship of resolution to other parameters can be expressed quantitatively by the following resolution equation:

$$R = \frac{1}{4}\sqrt{N} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_2}{k_2 + 1}$$

Where, N; represents the number of theoretical plates that are indicative for column efficiency, α; is the selectivity and k_1, k_2; are the capacity factors for two subsequent peaks. Clearly, decreasing N will greatly diminish resolution.

For isocratic analysis, flow rate has a significant effect on N, since efficiency is reduced, at high flow rate due the higher resistance to mass transfer (Van Deemter C term) [35]. The effect of flow rate on the efficiency of separation can be represented in plate height in µm as presented in Fig. (6). It can be noted that, column efficiency and back pressure are significantly affected by flow rate changes and that could explain the overlapping of ROS, BEZA or EZE and ATOR chromatograms at either higher or lower flow rates than 1.0 mL/min.In addition, broad and distorted chromatograms and longer retention times were obtained at lower flow rates, and therefore 1.0 mL/min was selected as the optimum flow rate for the investigated mixtures. Figure (2) presents resolution and sensitivities of the investigated drugs under the optimum chromatographic conditions.

CONCLUSION

A novel and rapid chromatographic method was developed for the simultaneous determination of antihyperlipidemics. This method offers for the first time advantage of simultaneous determination of six widely prescribed antihyperlidimics in a single chromatographic run. The developed method could be applied for estimating these drugs in pharmaceutical preparations and routine laboratory analysis.

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