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Validated Spectrophotometric Methods for Determination of Some Anti-Hyperlipidemic Used Drugs

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Abstract:

Present work describes accurate, precise, rapid and reproducible spectrophotometric methods for determination of Pravastatin (I), Simvastatin (II) and Ezetimibe (III), where (I) could be determined in presence of its acid-degradates by third derivative spectrophotometry, first derivative of ratio spectra and first derivative of pH-induced difference spectrophotometry, while (II) and (III) in binary mixtures could be simultaneously determined by first derivative of the ratio spectra. Also, (III) could be determined in presence of (II) by first-derivative spectrophotometry. All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied for determination of the studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the official and manufacturer's methods of analysis [for I and "II and III", respectively] and no significant differences were found.

Introduction:

Pravastatin sodium (I) and Simvastatin (II) are examples of statins that act by competitively inhibiting HMG-COA reductase enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis[1]. The ICH-guidelines[2] requires performing stress-testing of the drug substance that can help in identifying the likely degradation-products, also can be useful in establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used. Moreover, validated stability-indicating method should be applied in the stability study[3]. Stability-indicating methods can be specific one that evaluates the drug in the presence of its-degradation products, excipients and additives[4].

Most methods for (I) analysis utilized high performance liquid chromatographic techniques in biological fluids[5-13], thin layer chromatography [14], gas chromatography [15-16], capillary electrophoresis[17]and polarography[18] were reported. Different methods have been reported for determination of (II) including, spectrophotometric methods [19-20], high performance liquid chromatographic techniques [21-36] and gas chromatographic methods [37-39]. Ezetimibe (III) is the first in a new class of anti-hyperlipidemic drugs known as cholesterol absorption inhibitors. It blocks effectively intestinal absorption of dietary and biliary cholesterol [40]. Different methods used for (III) analysis using high performance liquid chromatographic techniques [41-43], high performance thin layer chromatographic technique [44] and spectrophotometric methods [45-46] were reported.

The main goal of this work is to establish accurate, precise, rapid and reproducible stability indicating spectrophotometric methods for the determination of (I) in the presence of its acid-degradates and for simultaneous determination of (II) and (III) in binary mixtures, which can be used for the routine quality control analysis of these drugs in raw material and pharmaceutical formulations and for stability studies.

Materials and Methods:

Chemicals and reagents

Pravastatin sodium was kindly supplied by Bristol- Mayers Squibb and certified to contain 99.99%. Lipostat® tablets: batch number: J42992, manufactured by Bristol-Mayers Squibb Company. Each tablet

was labeled to contain 20 mg of Pravastatin sodium. Simvastatin was kindly supplied by Amriya Pharmaceutical industries (Egypt) and certified to contain 99.95%. Ezetimibe was kindly supplied by Global Napi Pharmaceuticals (Egypt) and certified to contain 99.99%. Inegy® tablets: batch number: NE16760, manufactured by Global Napi Pharmaceuticals.

Each tablet was labeled to contain 20 mg of Simvastatin and 10 mg Ezetimibe.

Water (bi-distilled), Methanol (Reidel-de Häen, Sigma-Aldrich, Germany), Hydrochloric acid (BDH), aqueous 0.1M and Sodium hydroxide (BDH), aqueous 0.1M. All chemical and reagents used through this work are of spectroscopic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

Instruments

A double-beam Shimadzu (Japan) UV-VIS Spectrophotometer (UV-1601 PC), model TCC-240 A; connected to an IBM compatible computer and HP 695 C DeskJet printer is used. The bundled software is UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth is 0.2 nm and the wavelength scanning speed was 2800.0 $nmmin^{-1}$. The absorption spectra of the reference and the test solutions are recorded in1.0-ml quartz cells at 25.0 ^{0}C, using '$\Delta\lambda$ = 4 nm and scaling factor of 10 for computing first derivative (D^1)' and '$\Delta\lambda$ = 8 nm and scaling factor of 100 for third derivative (D^3)'.

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

Standard Solutions

Standard solutions of the studied drugs

Stock standard solutions of (I), (II) and (III), each having concentration of (1.0 $mg.ml^{-1}$) were prepared in water and methanol for (I) and (II and III), respectively, which were further diluted with the same solvents to obtain concentration (40 $\mu g.ml^{-1}$) of (I) and (100 $\mu g.ml^{-1}$) of (II & III) as working standard solutions, respectively.

Standard solution of Pravastatin acid-degradates

Standard solution of (I) acid-degradates was prepared by mixing 10 ml of the stock standard solution of (I) with 20 ml 0.1M HCl, heating in water-bath at 70^{o}C for 2 hours, cooling, neutralizing the media with 0.1M NaOH and making volume to 50 ml with water to obtain a concentration of 200 $\mu g.ml^{-1}$.

Complete dgradation was checked by using HPTLC system; silica gel 60 F_{254} plates and chloroform: ethanol: galacial acetic acid (9: 1: 0.2 v/v/v) as a developing system or HPLC system; Supelcosil C18 5 µm column and acetonitrile: acetic acid (pH 3.0) (50: 50 v/v) as a mobile phase.

Procedures:

Determination of Pravastatin sodium in presence of its acid-degradates:

Third derivative spectrophotometric method (D^3):

From standard working solution of (I), aliquots were transferred into a series of 10 ml volumetric flasks, diluted to the mark with methanol and scanned versus methanol. The first-derivative spectra (D^1) for (III) were computed, the amplitudes were recorded at 266.4 nm, the calibration graph having a concentration range of (8.0–36.0 μgml^{-1}) was constructed and the regression equation was then computed.

First derivative of ratio spectra method (DR^1):

Calibration curve was performed by transferring aliquots of (I) working standard solution into a series of 10 ml volumetric flasks, and diluting to volume with water to obtain a concentration range of 2–38 $\mu g.ml^{-1}$.

The spectrum of acid-degradate solution having concentration 2 μg.ml^{-1} was scanned and stored in the instrument PC as a devisor. The spectra of (I) were divided by the devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 250.7 nm, plotted versus concentrations, and the regression equation was computed.

First derivative of pH-induced difference spectrophotometric method (DD^1):

Aliquots of (I) working standard solution were transferred into two sets of 10 ml volumetric flasks, diluted with 0.1M HCl in the first set and with 0.1M NaOH in the second set, to obtain a concentration range of 2-22 μg.ml^{-1}. The absorption spectra of the first set were scanned against 0.1 M HCl and the second set against 0.1 M NaOH. The differences in the absorption spectra (ΔA) were determined and first derivative of ΔA spectra (DD^1) was then computed. The calibration curve was constructed by plotting the amplitudes at 255.4 nm versus concentrations, and the regression equation was then computed.

Determination of Simvastatin and Ezetimibe:

First-derivative of the ratio Spectra method (DR^1):

Into two series of 10 ml volumetric flasks, accurately measured volumes from (II) and (III) working standard solutions, were transferred, respectively, diluted to volume with methanol, and the absorption spectra were scanned versus methanol. The ratio spectra for (II) and (III) were constructed by dividing the absorption spectra of each drug over the normalized spectrum for 40 μg.ml^{-1} of the other drug and the first-derivative of the ratio-spectra (DR^1) were then computed. The amplitudes were recorded at '249.6 nm and 265.2 nm', the calibration graphs were constructed versus concentration, in a range of [(12.0–32.0 μg.ml^{-1}) and (8.0–28.0 μg.ml^{-1})] for (II) and (III), respectively.

First-derivative spectrophotometric method (D^1):

Accurate volumes of (III) working standard solution were transferred into series of 10 ml volumetric flasks, diluted to the mark with methanol and scanned versus methanol. The first-derivative spectra (D^1) for (III) were computed, the amplitudes were recorded at 266.4 nm, the calibration graph having a concentration range of (8.0–36.0 μgml^{-1}) was constructed and the regression equation was then computed.

Assay of the pharmaceutical preparations:

Twenty tablets of (Lipostat® and Inegy®) were individually weighed to get the average weight of the tablets and finely powdered, respectively. A sample of the powdered tablets, claimed to contain '20 mg' and '20 mg and 10 mg' of [(I) and (II and III mixture)] was transferred separately to 100 ml volumetric flasks, sonicated for 15 minutes with 50 ml of ['water for (I)' and of 'methanol for [(II) and (III)]', then the volume was brought to 100 ml with the same solvents and then filtered to prepare stock working solutions. Aliquots of the filtrate were further diluted with the same solvents to obtain a concentration of '10 μg.ml^{-1} for (I)' and of '20 μgml^{-1} and 10 μgml^{-1}of (II) and (III)' and then proceeds as described under (2.4.1 and 2.4.2), respectively.

Results and discussion:

Method development:

For Pravastatin sodium:

Third derivative spectrophotometric method (D^3):

The UV-spectra of (I) and its acid-degradates showed overlapping as shown in (Fig.1), which would not permit zero order determination of (I),

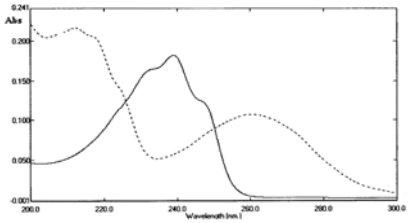


Fig.1: Zero order absorption spectra of Pravastatin sodium (—) and its acid-degradates (…), [4 µg.ml^{-1} each].

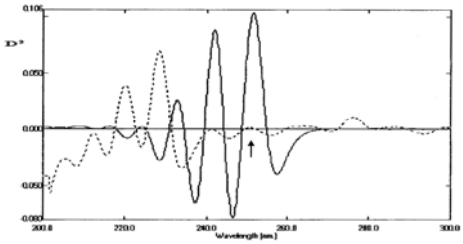


Fig.2: Third derivative spectra (D^3) of Pravastatin sodium (—) and its acid-degradates (…), [4 µg.ml^{-1} each].

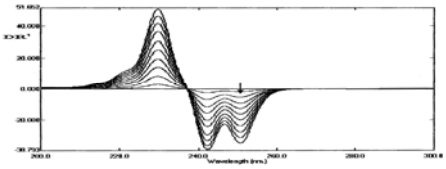


Fig.3: First derivative of ratio spectra (DR^1) for different concentrations (2-38 µg.ml^{-1}) of Pravastatin sodium at 250.7 nm, using 2.0 µg.ml^{-1} of its acid-degradates as a devisor.

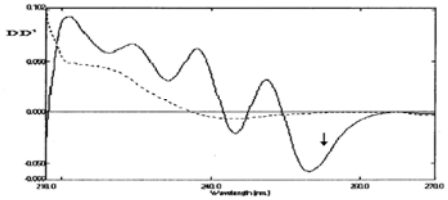


Fig.4: First derivative of difference spectra (DD^1) of Pravastatin sodium (−) and its acid-degradates (…), [4 µg.ml^{-1} each].

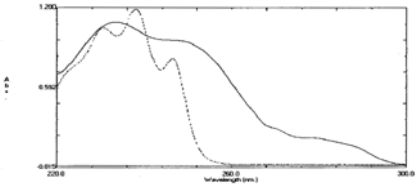


Fig.5: Zero order absorption spectra of Simvastatin (…) & Ezetimibe (−), [20.0 µg.ml^{-1} each].

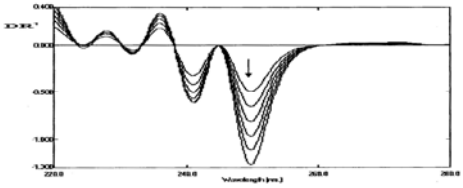


Fig. 6: First-derivative of ratio-spectra (DR^1) for different concentrations (12.0-32.0µg.ml^{-1}) of Simvastatin at 249.6 nm, using 40.0 µg.ml^{-1} of Ezetimibe as a devisor.

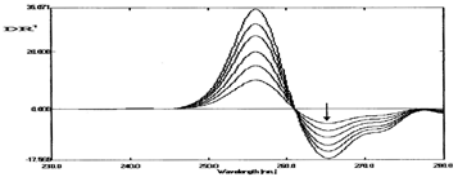


Fig. 7: First-derivative of ratio-spectra (DR^1) for different concentrations (8.0-28.0 µg.ml^{-1}) of Ezetimibe at 265.2 nm using 40.0 µg.ml^{-1} of Simvastatin as a devisor.

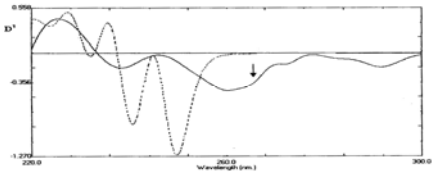
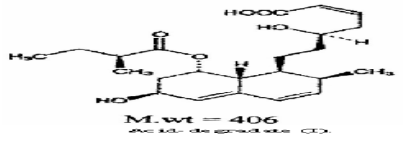


Fig. 8: First-derivative absorption spectra (D^1) of Ezetimibe (−) and Simvastatin (…), [20.0 µg.ml^{-1} each].



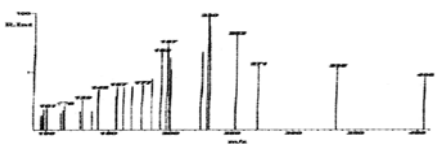


Fig. 9: Mass spectrum of the acid-degradate (I) of Pravastatin sodium.

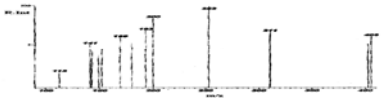


Fig.10: Mass spectrum of the acid-degradate (II) of Pravastatin sodium.

so derivative spectrophotometric methods were adopted, where zero-crossing point for acid-degradate of (I) was indicated. The third derivative spectrophotometric method (D^3) permitted a selective determination of (I) in the presence of its acid-degradates at 251.9 nm, as shown in (Fig. 2).

First derivative of ratio spectrophotometric method (DR^1):

The main advantage of derivative ratio spectra method (DR^n) might be the chance of taking measurement in correspondence to peaks and that the whole spectrum of interfering substance is cancelled, thus the wavelength selection for calibration is not critical. The main instrumental parameter conditions were optimized for a reliable determination of the compounds. Different divisor concentrations of acid-degradates were examined to select an appropriate concentration, which is very important factor in practice, where the best results were obtained by using 2 $\mu g.ml^{-1}$ concentration of acid-degradates standard working solution as a devisor. The first derivative of the ratio spectra (DR^1) at 250.7 nm permitted a selective shown in (Fig.3), where no noise was observed from the divisor.

First derivative of pH-induced difference spectrophotometric method (DD^1):

The change in the absorption spectrum of (I), by using acid and alkaline media could be used as a stability-indicating study. The direct UV measurement of ΔA spectra was not suitable for assaying (I) in presence of its acid-degradates due to severe overlapping, but computing the first derivative of ΔA spectra (DD^1) at 255.4 nm, allowing its determination as shown in (Fig.4), where zero-crossing point for its acid-degradates is indicated.

For Simvastatin and Ezetimibe:

The absorption spectra of (II) and (III) in methanol represented in (Fig.5) show severe overlapping, so direct UV absorption measurement for assaying this binary mixture seems to be impossible and consequently derivative (D^n) and derivative ratio-spectra (DR^n) were adopted to solve this problem.

First derivative of the ratio spectrophotometric method (DR^1):

The advantage of the derivative ratio spectral method may be the chance of doing measurement in correspondence of peaks, so there is a potential for greater sensitivity and accuracy. While the main disadvantages of zero-crossing method for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and the circumstance that the working wavelengths generally do not fall in correspondence of peaks of the spectrum. This particularly pronounced disadvantage when the slope of the spectrum is very high with consequent loss of accuracy and precision and the working wavelength is in proximity of the base of the spectrum, which causes poor sensitivity[47].

In this work, the ratio-spectra of serial standard working solutions of (II) were obtained by dividing their absorption spectra over a normalized spectrum of 40 μgml^{-1} concentration of (III) 'used as devisor', then the first-derivative of the ratio-spectra (DR^1) were computed at 249.6 nm, permitted a selective determination of (II) in the presence of (III) as shown in (Fig.6), where no noise was observed from the divisor. Similarly, (III) could be determined, by dividing their absorption spectra over a normalized spectrum for 40 $\mu g.ml^{-1}$ concentration of (II) 'used as a devisor', then the first-derivative of the ratio-spectra (DR^1) were computed at 265.2 nm, permitted a selective determination of (III) in the presence of (II) as shown in (Fig.7), where no noise was observed from the divisor.

Table 1: Validation report of the proposed spectrophotometric methods for the determination of Pravastatin sodium

Parameters	method		
	D^3	DR^1	DD^1
Linearity	6-38 µg.ml^{-1}	2-38 µg.ml^{-1}	2-22 µg.ml^{-1}
Slope	0.0252	0.9032	0.0097
Intercept	0.0145	0.0104	- 0.0022
Correlation coefficient (r)	0.9998	0.9999	0.9999
Accuracy[a]	99.90±0.895	99.94±0.552	99.90±0.690
Specificity[b]	99.72±1.201	99.76±0.628	100.33±0.860
Precision			
Repeatability[c] 'intra-day'	100.57±0.431	99.94±0.504	100.02±0.607
Intermediate precision[c] 'inter-day'	100.79±0.510	100.08±0.735	100.06±0.666

[a] Mean ± SD (D^3, n = 9; DR^1, n = 10; DD^1, n = 6); [b] Mean ± RSD% (n = 6), [c] Mean ± RSD% (n = 9)

Table 2: Validation report of the proposed spectrophotometric methods for the determination of Simvastatin and Ezetimibe.

Parameters	Simvastatin	Ezetimibe	
	[DR^1]	[DR^1]	[D^1]
	at 249.6 nm	at 265.2 nm	at 266.4 nm
Linearity (µgml^{-1})	12.0 – 32.0	8.0 – 28.0	8.0 – 36.0
slope	0.039	0.600	0.013
Intercept	0.036	0.239	0.007
Accuracy[a]	100.17±0.557	99.61±0.525	99.68±0.519
specificity[b]	100.42±0.387	100.17±0.736	100.06±0.578
Correlation coefficient (r)	0.9998	0.9996	0.9996
Precision			
Repeatability[c] 'intra-day'	100.09±0.270	99.80±0.716	99.69±0.537
Intermediate precision[c] 'inter-day'	100.07±0.416	99.89±0.935	99.54±0.620

[a] Mean ± SD (D^1, n = 8; DR^1, n = 6); [b] Mean ± R.S.D% (n = 6), [c] Mean ± R.S.D% (n = 9)

Table 3a: Statistical comparison between the proposed [D^1, DR^1 and DD^1] methods and the official BP method[48)*] for determination of Pravastatin sodium.

Parameters	Methods			
	D^3	DR^1	DD^1	Official Method*
Mean	99.90	99.40	99.94	99.75
S.D.	0.895	0.552	0.680	0.996
N	9	10	6	5
Variance	0.801	0.305	0.463	0.994
t-test	0.797 (2.16)	0.867 (2.20)	0.777 (2.23)	-
F-test	1.241 (3.84)	3.259 (3.63)	2.147 (5.19)	-

Values in parenthesis are the theoretical values of *t* and *F* at P = 0.05; *The official method is the BP HPLC method; C18 column, water: methanol: Glacial acetic acid: triethylamine (550: 450: 1: 1) as a mobile phase at flow rate 1.3 ml.mint^{-1}.

Table 3b: Statistical comparison between the proposed methods and the manufacturer method[49)*] for determination of Simvastatin and Ezetimibe.

Parameters	Methods				
	Simvastatin		Ezetimibe		
	DR^1	Manufacturer Method*	DR^1	D^1	Manufacturer Method*
Mean	100.17	99.88	99.61	99.68	100.46
S.D.	0.557	0.860	0.525	0.519	0.848
N	6	5	6	8	5
Variance	0.309	0.793	0.276	0.269	0.719
t-test	0.539 (2.23)	-	0.080 (2.23)	0.119 (2.18)	-
F-test	2.392 (5.19)	-	2.605 (5.19)	2.673 (4.12)	-

Values in parenthesis are the theoretical values of *t* and *F* at P = 0.05; *The manufacturer HPLC method obtained from Global Napi Pharmaceuticals; C18 column, acetonitrile: 25mM phosphate buffer [pH 4] (55: 45) as a mobile phase at flow rate 2 ml.mint^{-1}.

Table 4: Results of the laboratory prepared mixtures for Pravastatin sodium with its acid-degradates by the proposed spectrophotometric methods.

Sample no.	Pravastatin sodium µg.ml^{-1}	Acid-degradate µg.ml^{-1}	% Recovery *		
			D^3 251.9 nm	DR^1 250.7 nm	DD^1 255.4 nm
1	20.00	2.00	100.59	99.26	101.13
2	20.00	4.00	101.45	100.63	101.01
3	20.00	8.00	99.17	99.86	100.64
4	20.00	12.00	99.37	100.27	98.96
5	20.00	16.00	99.77	99.60	99.59
6	20.00	20.00	97.99	98.95	100.65
Mean			99.72	99.76	100.33
±R.S.D.%			±1.201	±0.628	±0.860

*Mean of three determinations

First derivative spectrophotometric method (D^1):

The first derivative (D^1) was suitable for determination of (III) in presence of (II), where the concentration of (III) was proportional to the amplitude at 266.4 nm, at which the absorbance of (II) equals to zero (zero crossing point), as shown in (Fig. 8).

Method Validation:

ICH guidelines[3)] for validation method were followed, where all validation parameters were shown in [Tables 1 and 2]. All the obtained results were statistically compared to the official[48)] and manufacturer's[49)] methods of [I and "II and III", analysis, respectively] and no significant differences were found [Tables 3a and 3b], respectively.

Specificity:

Degradation behavior of (I) was investigated by the proposed spectrophotometric methods, where (I) was determined in solutions containing different amounts of its acid-degradates by [D^3], [DR^1] and [DD^1] spectrophotometric methods. The Recovery % and R.S.D. % proved the high specificity of the adopted methods, where (I) could be determined in the presence of its acid-degradates (up to 50 %), as shown in [Table 4]. For (II) and (III), it was assessed by mixing known amounts of (II) and (III) as shown in [Table 5].

Standard addition technique:

To check the validity of the proposed

Table 5: Determination of Simvastatin and Ezetimibe in laboratory prepared mixtures by the proposed methods.

Mixtures no.	Simvastatin (µg.ml^{-1})	Ezetimibe (µg.ml^{-1})	%Recovery*		
			Simvastatin	Ezetimibe	
			DR^1 249.6 nm	DR^1 265.2 nm	D^1 266.4 nm
1	12	24	101.15	99.12	99.29
2	20	24	100.53	100.47	100.67
3	12	12	100.56	100.75	100.41
4	20	12	100.05	99.97	99.58
5	20	10	100.29	100.54	100.49
6	28	12	100.32	100.39	100.29
7	30	8	100.01	99.04	99.28
8	48	12	------	101.04	100.48
Mean			100.42	100.17	100.06
± R.S.D.			±0.385	±0.735	±0.578

*Mean of three determinations.

Table 6: Determination of Pravastatin sodium in pharmaceutical formulation[a] by the proposed spectrophotometric methods [D^3, DR^1 and DD^1] and application of standard addition technique.

Pharmaceutical formulation	Claimed	% Found ± SD*			Standard addition technique			
						%Recovery[b]		
		D^3	DR^1	DD^1	Added ($\mu g.ml^{-1}$)	D^3	DR^1	DD^1
Lipostat® tablets B.N: J42992[a]	20 mg	100.82 ± 0.105	100.72 ± 0.059	100.24 ± 0.276	6.00	98.94	98.70	99.04
					8.00	98.90	99.59	99.17
					10.00	101.02	100.46	101.03
					14.00	98.87	99.04	100.39
					18.00	100.3	99.62	99.86
Mean ± RSD%						99.61 ±0.999	99.48 ±0.672	99.89 ±0.836

[a]Lipostat® tablets (Batch no: J42992) (labeled to contain 20 mg Pravastatin sodium per tablet).
[b]Mean of three determinations.

methods, the standard addition method was applied by adding each drug to the previously analyzed tablets. The recovery of it was calculated determination of (I) in the presence of its acid-degradates as
by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of analysis of the commercial tablets and the standard addition method (recovery study) of (I) are shown in [Table 6] and of (II and III), are shown in [Tables 7a and 7b] suggested that there is no interference from any excipients, which are normally present in tablets.

Table 7a: Determination of Simvastatin in pharmaceutical formulation[a] by the proposed DR^1 method and application of Standard addition technique.

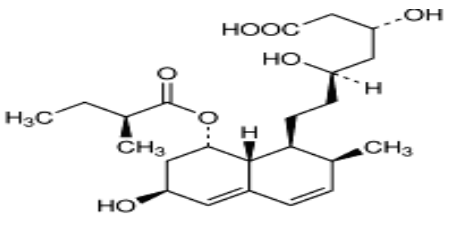
Pharmaceutical formulation	Claimed	% Found ± SD*	Standard addition technique	
			Added ($\mu g.ml^{-1}$)	%Recovery[b]
Inegy® tablets B.N: Ne 16760[a]	20 mg	99.41 ±0.349	12.00	99.63
			13.00	99.83
			14.00	100.56
			16.00	100.52
			18.00	99.36
Mean ± R.S.D%				99.78 ±0.753

Table 7b: Determination of Ezetimibe in pharmaceutical formulation[a] by the proposed [DR^1 and D^1] method and application of Standard addition technique.

Pharmaceutical formulation	Claimed	% Found ± SD*		Standard addition technique		
		D^1	DR^1	Added ($\mu g.ml^{-1}$)	%Recovery[b]	
					D^1	$^1DR^1$
Inegy® tablets B.N: Ne 16760[a]	10 mg	100.73 ± 0.403	100.70 ± 0.143	8.00	101.06	100.59
				9.00	99.04	98.89
				10.00	100.79	99.48
				14.00	100.85	99.79
				16.00	99.34	98.73
Mean ± R.S.D%					100.22 ±0.946	99.49 ±0.752

[a]Inegy® tablets (Batch no: Ne 16760) (labeled to contain 20 mg Simvastatin and 10 mg Ezetimibe per tablet); [b]Mean of three determinations.

Identification of acid-degradates of (I) by structure elucidation:



Pravastatin, M.wt = 446.5

(I) was influenced by the reaction with 0.1 M HCl for 2-hrs at 70°C, giving two acid-degradates (II) and (II). Degradate (I) is formed through dehydration of the secondary alcoholic-OH at C_3, because secondary and tertiary alcohols can easily undergo dehydration by acid-catalyzed elimination reaction[50]. While, degradate (II) is formed through intra-molecular esterification of (I) resulting in the lactone- form[51-53].

The identity of the acid-degradates was confirmed by separating these degradates on HPTLC plates using the developing system chloroform: ethanol: glacial acetic acid (9.0: 1.0: 0.2 v/v/v)] stated in the coming part, and then applying mass spectroscopy for each one. [Figures 9 and 10] show the parent peak at m/z 406 which is the molecular weight of each acid-degradate. These results confirm the proposed mechanism of the acid-degradation.

Conclusion:

The proposed methods are accurate, precise and specific ones, where the studied drugs (I), (II) and (III) can be determined in bulk powder and in pharmaceutical preparations without interference from common excipients present, also (I) can be determined in the presence of its acid-degradates and each of (II) and (III) can be simultaneously determined in binary mixtures. ICH guidelines were followed throughout the study for method validation and stress testing, and the suggested methods can be applied for routine quality control analysis and stability studies.

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