Arabian Journal of Chemistry (2011) 4, 299–305

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Kinetic spectrophotometric determination of pravastatin in drug formulations *via* derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)

Safwan Ashour [a,*], **Mouhammed Khateeb** [b]

[a] *Department of Chemistry, Faculty of Sciences, University of Aleppo, Aleppo, Syria*
[b] *Department of Chemistry, Faculty of Sciences, University of Al-Baath, Homs, Syria*

Received 25 March 2010; accepted 5 June 2010
Available online 30 June 2010

KEYWORDS

Pravastatin;
Kinetic spectrophotometry;
4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl);
Pharmaceutical dosage forms;
Method validation

Abstract A simple and sensitive kinetic spectrophotometric method for the quantitative analysis of pravastatin sodium (PVS) in pure and pharmaceutical formulations has been described. The method is based on the formation of colored product between PVS and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) in acetone medium at 55 ± 2 °C. The reaction is followed spectrophotometrically by measuring the increase in absorbance at 462 nm as a function of time. The initial rate and fixed time methods were adopted for constructing the calibration curves. The linearity ranges were found to be 15.0–50.0 and 10.0–70.0 μg mL^{-1} for initial rate and fixed time methods, respectively. The limits of detection for initial rate and fixed time methods are 0.029 and 0.086 μg mL^{-1}, respectively. Both methods have been applied successfully for the estimation of PVS in commercial dosage forms with no interference from the excipients. The results are compared with the HPLC pharmacopoeial method.

1. Introduction

Pravastatin is hexahydro-6-hydroxy-2-methyl-8-(2-methylbutyryloxy)-1-naphthyl-3,5-dihydroxyheptanoate, is one of a class of lipid-lowering compounds, the HMG-CoA reductase inhibitors, which reduce cholesterol biosynthesis. These compounds are used for the treatment of hypercholesterolemia. Pravastatin is characterized as one of the best, due to the hydroxyl group attached to its decal in ring, which results in a greater hydrophilicity than other HMG-CoA reductase inhibitors (Lennernäs and Fager, 1997; Hatanaka, 2000; Haria and McTavish, 1997).

Literature survey reveals that pravastatin is official in B.P. (British Pharmacopoeia, 2007). Several analytical methods are

* Corresponding author. Tel.: +963 933 604016.
E-mail address: profashour@myway.com (S. Ashour).

Peer review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2010.06.050

Production and hosting by Elsevier

available for the determination of the latter compound in pharmaceutical dosage forms, these include spectrophotometric method based on the reduction of ferric to ferrous ions followed by complexation with 2,2′-bipyridyl or 1,10-phenanthroline to produce orange red chromogen (Kalvikkarasi et al., 2009), high performance liquid chromatography (HPLC) with ultraviolet detector (Onal and Sagirli, 2006; Pasha et al., 2006; Ashour et al., 2008; Campos-Lara and Mendoza-Espinoza, 2008; Gomes et al., 2009), capillary electrophoresis (Kircali et al., 2004; Nigovic and Vegar, 2008), high performance liquid chromatography and capillary electrophoresis (Kocijan et al., 2005), high performance thin layer chromatography (Chaudhari et al., 2007), electroanalytical methods by square-wave adsorptive-stripping voltammetry (Nigovic, 2006), and differential plus polarography (Coskun et al., 1997). Pravastatin has been determined in biological samples by HPLC with laser-induced fluorescence detector (Dumousseaux et al., 1994) and ultraviolet detector (Iacona et al., 1994; Whigan et al., 1989; Bauer et al., 2005; Siekmeier et al., 2000; Otter and Mignat, 1998), liquid chromatography/tandem mass spectrometry (LC/MS/MS) (Zhu and Neirinck, 2003; Jemal et al., 1998; Mulvana et al., 2000), liquid chromatography/atmospheric-pressure chemical ionization mass spectrometry (LC/APCIMS) (Kawabata et al., 1998), and gas chromatography employing chemical ionization mass spectrometry (GC/CIMS) (Morris et al., 1993; Funke et al., 1989; Cai et al., 1996).

Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometeric methods. The literature is still poor in analytical assay methods based on kinetics for the determination of pravastatin in dosage forms. Some specific advantages that the kinetic methods possess are as follows (Perez-Bendito et al., 1996):

- Simple and fast methods because some experimental steps such as filtration, extraction, etc. are avoided prior to absorbance measurements.
- High selectivity since they involve the measurement of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.
- Other active compounds present in the commercial dosage forms may not interfere if they are resisting the chemical reaction conditions established for the proposed kinetic method.
- Colored and/or turbid sample background may possibly not interfere with the determination process.

Therefore, there is a need for another kinetic approach to estimate the drug in commercial dosage forms.

This paper describes a simple and sensitive kinetic spectrophotometric method for the determination of pravastatin in bulk and drug formulations. The method is based on the reaction between pravastatin and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in acetone medium resulting in the formation of yellow color, which absorbs maximally at 462 nm. The absorbance increases with time and therefore, two calibration procedures i.e., initial rate and fixed time methods are adopted for the determination of pravastatin in commercial dosage forms.

2. Experimental

2.1. Apparatus

A Jasco V-530 UV–vis spectrophotometer (Japan) with 1 cm quartz cells was used for all absorbance measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 375–550 nm and slit width 2 nm. Spectra were automatically obtained by Jasco system software. pH measurements were made with Consort C 830 (Belgium) with combined glass pH electrode. A water bath shaker (Grant Instruments, Cambridge Ltd., England) was used to control the heating temperature for color development.

2.2. Materials and reagents

Pravastatin sodium, PVS, ($C_{23}H_{35}O_7Na$, 446.52 g mol^{-1}) was supplied by CHEMLINE Healthcare (Lugano, Switzerland). Its purity was found to be 99.3% according to the HPLC method (British Pharmacopoeia, 2007). 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was purchase from Aldrich company. All other chemicals and reagents used were of analytical grade and all solutions were prepared with double distilled water.

2.3. Formulations

Pravastatin tablets supplied by Elsaad Pharma (Aleppo, Syria), each tablet was labeled to contain pravastatin sodium 20 or 40 mg and pravastin tablets supplied by Rasha (Aleppo, Syria), each tablet was labeled to contain pravastatin sodium 20 or 40 mg.

2.4. Solutions

Standard stock solution of pravastatin sodium of 0.5 mg mL^{-1} was prepared in 100 mL volumetric flask by dissolving the required amount of pravastatin sodium with 40 mL methanol, the volume was then diluted to the mark with acetone. The working standard solutions were freshly prepared by suitable dilution of the stock solution with acetone. NBD-Cl 0.2% solution was freshly prepared with acetone.

2.5. General procedures

2.5.1. Initial rate method

Aliquots of standard PVS solution (0.30–1.00 mL, 0.5 mg mL^{-1}) were transferred into a series of 10 mL calibrated volumetric flasks. Then 0.50 mL of NBD-Cl solution was added and the volume was made up to the mark with acetone, mixed well and heated on a water bath at 55 ± 2 °C. After mixing, the contents of each flask were immediately transferred to the spectrophotometric cell and the increase in absorbance was recorded at 462 nm as a function of time between 0–5 min against reagent blank treated similarly. The initial rate of the reaction (v) at different concentrations was obtained from the slope of the tangent to the absorbance–time curve. The calibration curve was constructed by plotting the logarithm of the initial rate (log v) *vs* the logarithm of the molar

concentration of the PVS (log *C*). The amount of the drug was obtained either from the calibration graphs or the regression equation.

2.5.2. Fixed time method

Aliquots of standard PVS solution (0.20–1.40 mL, 0.5 mg mL^{-1}) were transferred into a series of 10 mL calibrated volumetric flasks. Then 0.50 mL of NBD-Cl solution was added and the volume was made up to the mark with acetone, mixed well and heated on a water bath at 55 ± 2 °C for 25 min. After mixing, the contents of each flask were immediately transferred to the spectrophotometric cell and the absorbance was recorded at 462 nm against reagent blank treated similarly. The calibration curve was constructed by plotting the absorbance against the final concentration of the drug. The amount of the drug in each sample was computed either from calibration curve or regression equation.

2.6. Procedure for formulations

Twenty tablets containing PVS were weighed and finely powdered. An amount of the powder equivalent to 25 mg of PVS was dissolved in a 15 mL of methanol, mixed for about 5 min and then filtered through Whatman filter paper number 40. The volume of filtrate was adjusted to 20 mL with methanol and diluted in a 50 mL volumetric flask to the volume with acetone to achieve a concentration of 0.5 mg mL^{-1}. The general procedures were then followed in the concentration ranges mentioned above.

3. Results and discussion

3.1. Absorption spectra

Several pharmaceutical compounds have been determined through derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), such as, acetylcysteine, carbocisteine and captopril (Taha et al., 2008; Haggag et al., 2008). In the present study, PVS was found to react with NBD-Cl in acetone medium at 55 ± 2 °C producing a yellow color with maximum absorbance at 462 nm (Fig. 1).

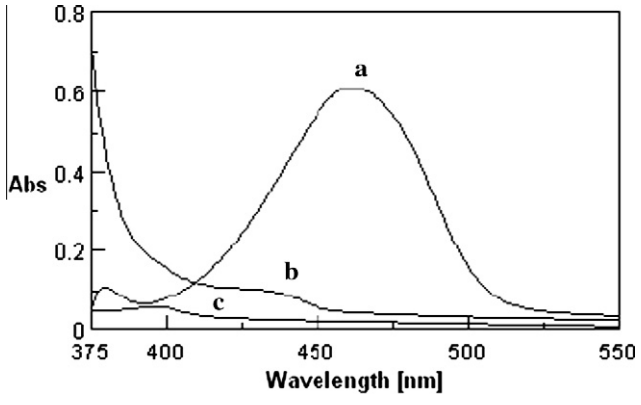


Figure 1 Absorption spectra of (a) 50 μg mL^{-1} PVS + 0.50 mL NBD-Cl 0.2% against reagent blank, (b) reagent blank (NBD-Cl 0.2%) against acetone, and (c) 50 μg mL^{-1} of PVS against acetone.

3.2. Optimization of reaction conditions

The optimum conditions for the development of method were established by varying the parameters one at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as solvents, temperature, concentration of NBD-Cl and time of heating were studied.

The pure drug is soluble in water, methanol, ethanol and acetone. The colored product between PVS and NBD-Cl is not formed in water medium and no problem was found when PVS is dissolved in methanol, ethanol or acetone. Acetone was found to be the best solvent for formation of colored product. Whereas, in the application of the method, if a quantity of tablets containing pravastatin sodium was dissolved in methanol, ethanol or acetone, mixed, filtered and then was diluted to the volume with the solvent to achieve a required concentration of PVS, the turbid was formed in the solution. To resolve this problem, many solvents were used and 40% methanol/acetone (*v*/*v*) was the best ratio to prepare the sample of formulations. So, the same ratio of solvents was used to prepare the standard stock solution and blank has the same ratio of solvents. The working solutions were prepared by diluting very small volumes of stock solution with acetone, so the ratio of methanol is negligible.

When using acidic, neutral or basic buffer media, such as britton and borate buffers, reagent forms an orange yellow color. This will decrease the absorbance of the sample solution when using it as blank.

The effect of temperature on the reaction was studied in the range of 20–65 °C. About 55 °C was found to be optimal for maximum color development.

The most important factor affecting on the formation of yellow product was the concentration of NBD-Cl. Fig. 2 shows that 0.50 mL of 0.2% w/v NBD-Cl solution gave maximum sensitivity. Increasing the volume of NBD-Cl leads to decrease in the absorbance; this may be due to the high background absorbance of the reagent.

The influence of the time of heating was investigated in the range of 5–60 min. The experimental results show that heating in the range 5–35 min gave the optimal values in kinetic studies.

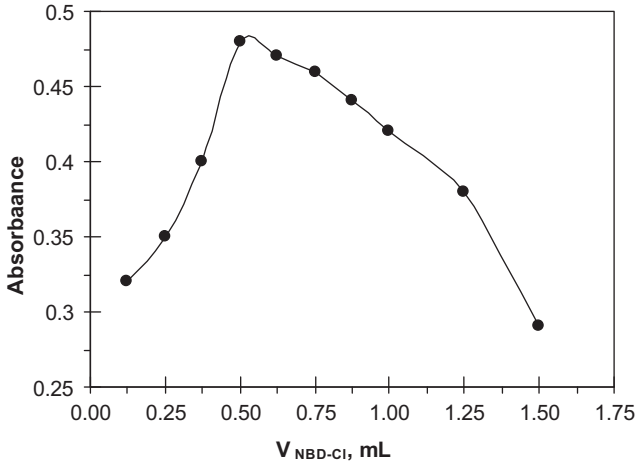


Figure 2 Effect of concentration of NBD-Cl on the formation of colored product PVS–NBD-Cl, [PVS] = 50 μg mL^{-1} at 55 °C for 10 min.

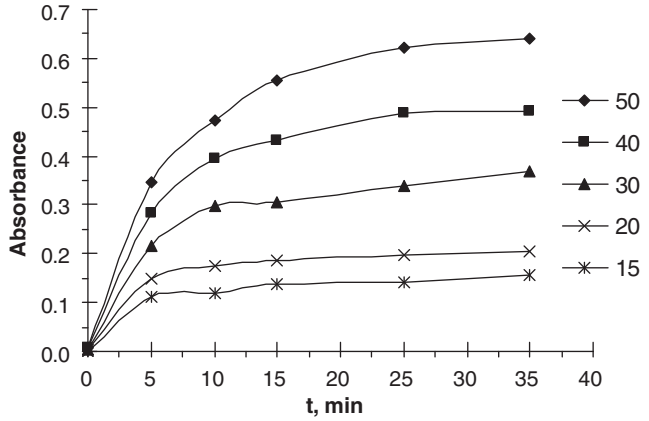


Figure 3 Absorbance–time curve for the reaction of PVS with NBD-Cl; [PVS] = 15–50 μg mL^{-1}.

The color product was stable for at least two days at room temperature.

3.3. Quantitation methods

Because the intensity of the color increased with time (Fig. 3), this was used as the basis for a useful kinetic method for the determination of PVS. The initial rate, rate constant, variable time (fixed concentration or fixed absorbance) and fixed time methods (Kopanica et al., 1983; Perez-Bendito and Silva, 1988) were tested and the most suitable analytical methods were chosen regarding the applicability, sensitivity and the values of the intercept and correlation coefficient (R^2).

3.3.1. Initial rate method

The initial rate of reaction would follow a pseudo order rate constant and obeyed the following rate equation:

$$v = \Delta A/\Delta t = k'C^n$$

where v is the reaction rate, A is the absorbance, t is the measuring time, k' is the pseudo order rate constant, C is the concentration of the drug in mol L^{-1} and n is the order of the reaction. A calibration curve was constructed by plotting the logarithm of the initial rate of reaction ($\log v$) *vs* logarithm of drug concentration ($\log C$) which showed a linear relationship over the concentration range of 15–50 μg mL^{-1} (Fig. 4).

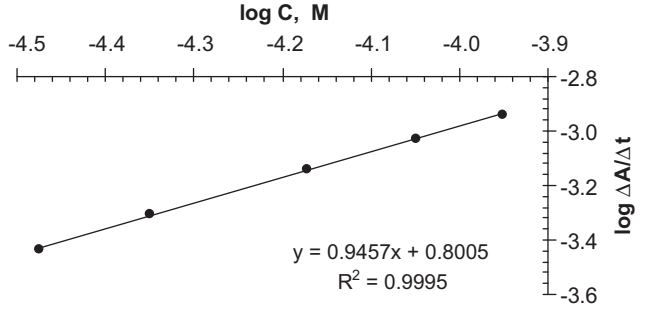


Figure 4 Calibration plot of logarithm rate of the reaction against logarithm molar concentration of PVS for initial rate method.

The logarithmic form of the above equation was written as follows:

$$\log v = \log \Delta A/\Delta t = \log k' + n \log C$$

$$\log v = \log \Delta A/\Delta t = 0.8005 + 0.9457 \log[\text{PVS}] \quad (R^2 = 0.9995)$$

Thus, $k' = 6.32\ \text{S}^{-1}$, and the reaction is the first order ($n = 0.9457$) with respect to PVS concentration. The limit of detection (LOD) and limit of quantification (LOQ) for initial rate method were determined and were found to be 0.029 and 15 μg mL^{-1}, respectively.

3.3.2. Rate constant method

Graphs of log absorbance *vs* time for PVS concentration in the range of 15–50 μg mL^{-1} (3.36×10^{-5} to 11.19×10^{-5} M) were plotted and all appeared to be rectilinear. Pseudo order rate constant (k') corresponding to different PVS concentrations were calculated from the slopes multiplied by −2.303 and are presented in Table 1. Regression of C *vs* k' gave the following equation:

$$k' = -0.00011 - 1.777C \quad (R^2 = 0.8779)$$

3.3.3. Variable time method

Reaction rate data were recorded for different PVS concentrations in the range 15–50 μg mL^{-1}. A preselected value of the absorbance 0.34 was fixed and the time was measured in the seconds (Table 2). The reciprocal of time ($1/t$) *vs* the initial concentration of PVS was plotted and the following equation of calibration graph was obtained:

$$1/t = -0.0032 + 59.536C \quad (R^2 = 0.9735)$$

The range of PVS concentrations giving the most satisfactory results was limited 30–50 μg mL^{-1} (6.72×10^{-5} to 11.19×10^{-5} M).

3.3.4. Fixed time method

At preselected fixed time, the absorbance of yellow colored solution containing varying amounts of PVS was measured at 55 °C and 462 nm. Calibration graphs were constructed by plotting the absorbance against the initial concentration of

Table 1 Values of rate constant k'.

[PVS] (M)	k' (S^{-1})
3.36×10^{-5}	-1.8×10^{-4}
4.48×10^{-5}	-1.6×10^{-4}
6.72×10^{-5}	-2.5×10^{-4}
8.96×10^{-5}	-2.8×10^{-4}
11.19×10^{-5}	-3.0×10^{-4}

Table 2 Values of reciprocal time taken at fixed absorbance for the different rates of variable concentration of PVS at constant concentrations of NBD-Cl.

[PVS] (M)	$1/t$ (S^{-1})
6.72×10^{-5}	6.67×10^{-4}
8.96×10^{-5}	23.81×10^{-4}
11.19×10^{-5}	33.33×10^{-4}

Table 3 Regression equations for PVS at fixed time and 55 °C.

Time (min)	Regression equation	Correlation coefficient	Linear range (μg mL^{-1})
5	$A = 0.0067C + 0.0127$	0.9993	15–50
10	$A = 0.0102C - 0.0244$	0.9926	15–50
15	$A = 0.0119C - 0.0457$	0.9991	15–60
25	$A = 0.0138C - 0.0699$	0.9996	10–70
35	$A = 0.0145C - 0.0745$	0.9982	10–70

A, absorbance; C, concentration.

Table 4 Analytical characteristics of the fixed time (25 min) method.

Parameters	PVS
λ_{max} (nm)	462
Beer's law limit (μg mL^{-1})	10–70
Molar absorptivity (L mol^{-1} cm^{-1})	0.49×10^4
Stoichiometric relationship, PVS:NBD-Cl	1:1
Logarithmic formation constant	6.02
Optimum photometric range (μg mL^{-1})	20–50
Detection limit (μg mL^{-1})	0.086
Limit of quantification (μg mL^{-1})	10
Sandell's sensitivity (μg cm^{-2} per 0.001 absorbance unit)	0.182
Regression equation[a]	$A = 0.0138C - 0.0699$
Correlation coefficient, r	0.9996

[a] $A = mC + b$, where C is the concentration in μg mL^{-1} and A is the absorbance

PVS at fixed time 5, 10, 15, 25 and 35 min. The regression equations, correlation coefficients and linear ranges are given in (Table 3). Correlation coefficient, intercept and slope values for the calibration data calculated using the least squares method (Miller and Miller, 1993).

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient, linear range and the intercept were obtained for a fixed time of 25 min. Therefore, the fixed time of 25 min was utilized for the assay of PVS concentration. The limit of detection (LOD) and limit of quantification (LOQ) for fixed time (25 min) method were determined and found to be 0.086 and 10 μg mL^{-1}, respectively. For more accurate analysis, Ringbom optimum concentration range was calculated to be 20–50 μg mL^{-1}. Table 4 shows the values of molar absorptivity, Sandell's sensitivity and some analytical characteristics for fixed time (25 min) method.

3.4. Stoichiometric relationship

The composition of colored product was determined by Job's method of continuous variation and mole-ratio method (Rose, 1964), for fixed time (25 min) method. It is apparent from the data that a molar ratio of 1:1 PVS to NBD-Cl.

As result, the most acceptable values of the correlation coefficients were obtained for an initial rate and fixed time (25 min) methods. Thus, they were used for the determination of PVS in pure form and pharmaceutical formulations.

Table 5 Accuracy and precision for the determination of PVS in bulk powder by the proposed methods (initial rate and fixed time).

Method	PVS (μg mL^{-1})		Er%	RSD (%)	%Recovery ± SD
	Taken	Found[a]			
Initial rate	20.00	20.06	0.30	1.05	100.30 ± 1.05
	30.00	30.12	0.40	0.88	100.40 ± 0.88
	40.00	40.18	0.45	0.81	100.45 ± 0.81
	50.00	50.48	0.96	0.83	100.96 ± 0.84
Fixed time	10.00	10.13	1.30	1.38	101.30 ± 1.40
	30.00	30.15	0.50	0.95	100.50 ± 0.95
	50.00	50.21	0.42	0.35	100.42 ± 0.35
	70.00	70.15	0.21	0.33	100.21 ± 0.33

[a] Average of six determinations.

3.5. Analytical methods validation

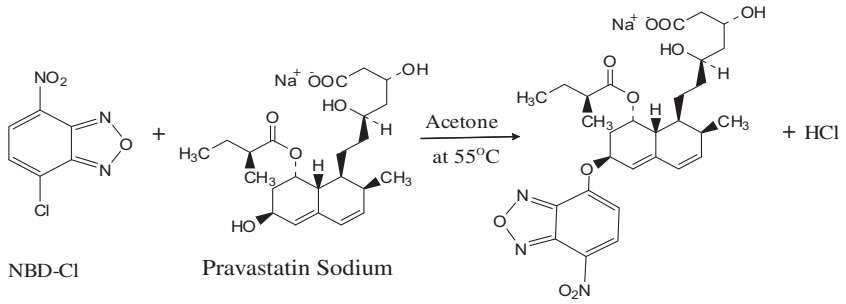
The accuracy and precision of the proposed methods were carried out by six determinations at four different concentrations. Percentage relative standard deviation (RSD%) as precision and percentage relative error (Er%) as accuracy of the suggested methods were calculated. Table 5 shows the values of relative standard deviations for different concentrations of the PVS determined from the calibration curves. These results of accuracy and precision show that the proposed methods

Table 6 Application of the proposed methods to the determination of PVS in dosage forms.

Sample	%Recovery[a] ± SD		
	Proposed methods		Official method
	Initial rate	Fixed time	
Pure PVS	100.53 ± 0.88	100.61 ± 0.74	99.40 ± 0.82
t-Value	1.36	1.84	
F-Value	1.15	1.23	
Pravastatin tablets (20 mg)			
X ± SD[a]	100.27 ± 0.60	100.35 ± 0.82	100.80 ± 0.76
t-Value[b]	1.05	0.94	1.32
F-Value[b]	1.60	1.16	
Pravastatin tablets (40 mg)			
X ± SD[a]	100.32 ± 0.72	100.70 ± 1.05	101.05 ± 1.20
t-Value[b]	0.99	1.52	1.28
F-Value[b]	2.78	1.31	
Pravastin tablets (20 mg)			
X ± SD[a]	100.67 ± 0.62	101.25 ± 1.14	99.89 ± 1.00
t-Value[b]	2.50	2.44	1.79
F-Value[b]	2.60	1.30	
Pravastin tablets (40 mg)			
X ± SD[a]	101.34 ± 1.20	100.36 ± 0.95	100.52 ± 0.70
t-Value[b]	2.49	0.83	1.81
F-Value[b]	2.94	1.84	

[a] Five independent analyses.

[b] Theoretical values for t and F-values at five degree of freedom and 95% confidence limit are (t = 2.776) and (F = 6.26).



Scheme 1 The proposed pathway of the reaction between PVS and NBD-Cl.

have good repeatability and reproducibility. The proposed methods were found to be selective for the estimation of PVS in the presence of various tablet excipients. For this purpose, a powder blend using typical tablet excipients was prepared along with the drug and then analyzed. The recoveries were not affected by the excipients and the excipients blend did not show any absorption in the range of analysis.

3.6. Application to the pharmaceutical dosage forms

The performance of the proposed methods was assessed by comparison with the pharmacopoeial method (British Pharmacopoeia, 2007). Mean values were obtained with a Student's *t*- and *F*-tests at 95% confidence limits for four degrees of freedom. The results showed comparable accuracy (*t*-test) and precision (*F*-test), since the calculated values of *t*- and *F*-tests were less than the theoretical data.

The proposed procedures were applied to determine PVS in its pharmaceutical formulations. The results in Table 6 indicate the high accuracy and precision. As can be seen from Table 6, the proposed methods have the advantages of being virtually free from interferences by excipients such as glucose, magnesium stearate, lactose, and starch or from common degradation products. The results obtained were compared statistically by the Student's *t*-test (for accuracy) and the variance ratio *F*-test (for precision) with those obtained by the official method for the samples of the same batch (Table 6). The values of *t*- and *F*-tests obtained at 95% confidence level did not exceed the theoretical tabulated value indicating no significant difference between the methods compared.

3.7. Mechanism of the color reaction

A nitro group in NBD-Cl formula reduces the ring activity, especially at para position, so the pair of electron at oxygen in PVS (at meta position) bounds to para position in NBD-Cl formula forming a colored product. Therefore, hydrochloride acid has been formed and the ring returns to its aromaticity. The formation of HCl is not indicate that the reaction is pH dependent, because the amount of HCl produced is very small and diluted. The reaction mechanism is shown in Scheme 1.

4. Conclusion

The proposed kinetic spectrophotometric methods for the determination of PVS were simple, rapid, accurate and precise and hence can be used for the routine analysis of PVS in bulk and pharmaceutical formulations. 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was used as reagent in acetone medium. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients in the estimation.

References

Ashour, S., Nakshbandi, H., Omar, S., 2008. Quantitative determination of pravastatin in pharmaceutical dosage forms by high-performance liquid chromatography with ultraviolet detection. Int. J. Biomed. Sci. 4, 135.

Bauer, S., Mwinyi, J., Stoeckle, A., Gerloff, T., Roots, I., 2005. Quantification of pravastatin in human plasma and urine after solid phase extraction using high performance liquid chromatography with ultraviolet detection. J. Chromatogr. B 818, 257.

British Pharmacopoeia, 2007. Her Majesty's Stationery Office Ltd., London.

Cai, K.H., Tan, B.Y., Feng, Z.Y., Li, Z.W., Huang, M., Zhao, X.L., 1996. The quantitative determination of pravastatin in plasma by a gas chromatography-mass spectrometry method. Sepu 14, 121.

Campos-Lara, M., Mendoza-Espinoza, J.A., 2008. Development of a selective extraction method for pravastatin quantification in tablets using HPLC with ultraviolet detection. J. Liq. Chromatogr. Relat. Technol. 31, 619.

Chaudhari, B.G., Patel, N.M., Shah, P.B., 2007. Determination of simvastatin, pravastatin sodium and rosuvastatin calcium in tablet dosage forms by HPTLC. Indian J. Pharm. Sci. 69, 130.

Coskun, N.Y., Aycan, S., Sungur, S., 1997. Differential pulse polarographic determination of pravastatin sodium in tablets. Pharmazie 52, 485.

Dumousseaux, C., Muramatsu, S., Takasaki, W., Takahagi, H.J., 1994. Highly sensitive and specific determination of pravastatin sodium in plasma by high-performance liquid chromatography with laser-induced fluorescence detection after immobilized antibody extraction. Pharm. Sci. 83, 1630.

Funke, P.T., Ivashkiv, E., Arnold, M.E., Cohen, A.I., 1989. Determination of pravastatin sodium and its major metabolites in human serum and plasma by capillary gas chromatography-negative-ion chemical-ionization mass spectrometry. Biomed. Environ. Mass Spectrom. 18, 904.

Gomes, F.P., Garcia, P.L., Alves, J.M.P., Singh, A.K., Kedor-Hackmann, E.R.M., Santoro, M.I.R.M., 2009. Development and validation of stability-indicating HPLC methods for quantitative determination of pravastatin, fluvastatin, atorvastatin, and rosuvastatin in pharmaceuticals. Anal. Lett. 42, 1784.

Haggag, R., Belal, S., Shaalan, R., 2008. Derivatization with 4-chloro-7-nitro-2,1,3-benzoxadiazole for the spectrophotometric and differential pulse polarographic determination of acetylcysteine and captopril. Sci. Pharm. 76, 33.

Haria, M., McTavish, D., 1997. Pravastatin. A reappraisal of its pharmacological properties and clinical effectiveness in the management of coronary heart disease. Drugs 53, 299.

Hatanaka, T., 2000. Clinical pharmacokinetics of pravastatin: mechanisms of pharmacokinetic events. Clin. Pharmacokinet. 39, 397.

Iacona, I., Regazzi, M.B., Buggia, I., Villani, P., Fiorito, V., Molinaro, M., Guarnone, E., 1994. High-performance liquid chromatographic determination of pravastatin in plasma. Ther. Drug Monit. 16, 191.

Jemal, M., Qing, Y., Whigan, D.B., 1998. The use of high-flow high performance liquid chromatography coupled with positive and negative ion electrospray tandem mass spectrometry for quantitative bioanalysis via direct injection of the plasma/serum samples. Rapid Commun. Mass Spectrom. 12, 1389.

Kalvikkarasi, S., Vaidhyalingam, V., Niraimathi, V., Aruna, A., 2009. Spectrophotometric determination of pravastatin sodium in pharmaceutical oral solid dosage forms. Asian J. Chem. 21, 1648.

Kawabata, K., Matsushima, N., Sasahara, K., 1998. An automated method for the simultaneous determination of pravastatin and its main metabolite in human plasma by high-performance liquid chromatography-atmospheric-pressure chemical ionization mass spectrometry. Biomed. Chromatogr. 12, 271.

Kircali, K., Tunçel, M., Aboul-Enein, H.Y., 2004. Determination of pravastatin in tablets by capillary electrophoresis. Farmaco 59, 241.

Kocijan, A., Grahek, R., Bastarda, A., Zupancic Kralj, L., 2005. Fast analysis of pravastatin in production media. J. Chromatogr. B 822, 311.

Kopanica, M., Satra, V., Echschlager, K., Rorsak, I., Koduys, Z., Sandr, Z.K., 1983. Kinetic Methods in Chemical Analysis. Elsevier, Amsterdam, The Netherlands, 25p.

Lennernäs, H., Fager, G., 1997. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Clin. Pharmacokinet. 32, 403.

Miller, J.C., Miller, J.N., 1993. Statistics in Analytical Chemistry, 3rd ed., Ellis Horwood, Chichester.

Morris, M.J., Gilbert, J.D., Hsieh, J.Y.K., Matuszewski, B.K., Ramjit, H.G., Bayne, W.F., 1993. Determination of the HMG-CoA reductase inhibitors simvastatin, lovastatin and pravastatin in plasma by gas chromatography-chemical-ionization mass spectrometry. Biol. Mass Spectrom. 22, 1.

Mulvana, D., Jemal, M., Pulver, S.C., 2000. Quantitative determination of pravastatin and its biotransformation products in human serum by turbo ion spray LC/MS/MS. J. Pharm. Biomed. Anal. 23, 851.

Nigovic, B., 2006. Electrochemical properties and square-wave voltammetric determination of pravastatin. Anal. Bioanal. Chem. 384, 431.

Nigovic, B., Vegar, I., 2008. Capillary electrophoresis determination of pravastatin and separation of its degradation products. Croat. Chem. Acta 81, 615.

Onal, A., Sagirli, O., 2006. Development of a selective LC method for the determination of pravastatin sodium. Chromatographia 64, 157.

Otter, K., Mignat, C., 1998. Determination of pravastatin in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B. 708, 235.

Pasha, M.K., Muzeeb, S., Basha, S.J.S., Shashikumar, D., Mullangi, R., Srinivas, N.R., 2006. Analysis of five HMG-CoA reductase inhibitors- atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin: pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and in vitro metabolism studies. Biomed. Chromatogr. 20, 282.

Perez-Bendito, D., Silva, M., 1988. Kinetic Methods in Analytical Chemistry. John Wiley and Sons, New York, NY, Chapter 11, 44p.

Perez-Bendito, D., Gomez-Hens, A., Silva, M., 1996. Advances in drug analysis by kinetic methods. J. Pharm. Biomed. Anal. 14, 917.

Rose, J., 1964. Advanced Physico-chemical Experiments. Pittman, London, p. 54.

Siekmeier, R., Gross, W., Marz, W., 2000. Determination of pravastatin by high-performance liquid chromatography. Int. J. Clin. Pharmacol. Ther. 38, 419.

Taha, E.A., Hassan, N.Y., Abdel Aal, F., Abdel Fattah, L.E., 2008. Kinetic spectrophotometic determination of acetylcysteine and carbocisteine in bulk powder and in drug formulations. Sci. Asia 34, 107.

Whigan, D.B., Ivashkiv, E., Cohen, A.I., 1989. Determination of pravastatin sodium and its isomeric metabolite in human urine by HPLC with UV detection. J. Pharm. Biomed. Anal. 7, 907.

Zhu, Z.M., Neirinck, L., 2003. High performance liquid chromatography coupled with negative ion tandem mass spectrometry for determination of pravastatin in human plasma. J. Chromatogr. B 783, 133.