Chem. Anal. (Warsaw), **52**, 771 (2007)

Comparative Validation of Densitometric and Videodensitometric Determination of Lovastatin and Simvastatin in Pharmaceuticals

by Łukasz Komsta*, Robert Skibiński, Hanna Hopkała and Małgorzata Winiarczyk-Serwacka

Department of Medicinal Chemistry, Skubiszewski Medical University, ul. Jaczewskiego 4, 20-090 Lublin, Poland,

Keywords: Simvastatin; Lovastatin; Densitometry; Videodensitometry; Pharmaceuticals

Densitometric and videodensitometric methods for determination of lovastatin and simvastatin in commercially available pharmaceuticals have been described. Analysis was performed using HPTLC Si F254 plates and hexane–methylethylketone (55:45 v/v) mobile phase. Densitometric detection was performed at 230 nm, and videoscanning at 254 nm. Calibration plots were constructed in the range 4–16 μg per spot for both drugs. Calibration data were subjected to statistical analysis using AIC criterion. Quadratic regression was finally chosen. Active substances were extracted from tablets with methanol. Densitometric method was much more precise than videodensitometric one. However, no significant differences in the accuracy between both procedures were observed. The proposed methods can be used in routine drug analysis due to their precision, accuracy, and relatively low consumption of materials and reagents.

W pracy opisano densytometryczną i wideodensytometryczną metodę oznaczania lowastatyny i simwastatyny w dostępnych na rynku polskim preparatach farmaceutycznych. Analizę przeprowadzono na płytkach HPTLC pokrytych żelem krzemionkowym z użyciem fazy ruchomej o składzie heksan–metyletylketon (55:45 v/v). Detekcję densytometryczną przeprowadzono przy długości fali 230 nm, a wideoskanowanie przy 254 nm. Krzywe kalibracyjne skonstruowano w zakresie stężeń 4–16 μg na plamkę. Dane kalibracyjne zostały poddane analizie statystycznej i na podstawie kryterium AIC wybrano regresję kwadratową. Ekstrakcję z tabletek przeprowadzono metanolem. Pomiędzy obiema metodami nie stwierdzono różnic w dokładności, natomiast densytometria odznaczała się wyraźnie lepszą precyzją. Proponowana metoda jest dokładna i precyzyjna, może być użyta w rutynowej analizie produktów leczniczych.

* Corresponding author. E-mail: lukasz.komsta@am.lublin.pl

Statins are widely used now in the treatment of different forms of hypercholesterolemia and hypertriglyceridemia. They exhibit different chemical structures. They block cholesterol synthesis by inhibiting HMG-CoA reductase. Two commonly used statins: lovastatin ((2S)-2-methylbutanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro--3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1--naphthalenyl ester) and simvastatin (2,2-dimethylbutanoic acid (1S,3R,7S,8S,8aR)--1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H--pyran-2-yl]ethyl]-1-naphthalenyl ester) are studied in this paper.

Several methods of determination of lovastatin and simvastatin in pharmaceuticals have been already reported. They include: HPLC [1–4], derivative spectrophotometry [5–7], MEKC [8], and and polarography [9]. European and United States Pharmacopoeias suggest elaboration of new determination methods in order to reduce consumption of reagents and materials. This requirement is related to environmental pollution and harmful impact on human health of currently used chemicals. HPTLC method combined with either densitometry or videodensitometry is highly efficient in comparison to HPLC, because many samples can be run simultaneously using a very small amount of solvents.

The purpose of this paper was to investigate the possibilities of quantitative analysis of simvastatin and lovastatin in pharmaceuticals by HPTLC combined with either densitometry or videodensitometry. The proposed methods were validated and compared.

EXPERIMENTAL

Materials

Simvastatin and lovastatin were kindly donated by Polfa–Grodzisk (Grodzisk, Poland). Commercially available pharmaceuticals: Liprox (20 mg of lovastatin, Biofarm, Poznań, Poland), Lovasterol (40 mg of lovastatin, Polpharma, Starogard, Poland), Lovastin (20 mg of lovastatin, Polfa, Grodzisk, Poland), Simvachol (20 mg of simvastatin, Polfa, Grodzisk, Poland), Simvahexal (20 mg of simvastatin, Hexal AG, Warsaw, Poland), and Simvasterol (40 mg of simvastatin, Polpharma, Grodzisk, Poland) were purchased in the local pharmacy.

Analytical grade methanol (PoCH, Gliwice, Poland) was used for extraction. Methylethylketone (JTBaker, UK) and hexane (Merck, Germany) were of HPLC grade.

Procedures

All analyses were performed on F254 HPTLC silica gel plates (Merck, Germany) using hexane––methylethylketone (45:55 v/v) mobile phase. Plates were developed at a distance of 4.5 cm in horizontal DS chambers (Chromdes, Lublin, Poland). Spots were applied using a Desaga AS–30 applicator equipped with a 100 µL-in-volume syringe.

Densitometric scanning was performed on a Desaga CD–60 densitometer at 230 nm using reflectance mode, slit height 0.4 mm, slit width 4 mm. Peak areas were used for calculations.

Videodensitometry was performed using a Desaga VD–40 videoscanner with standard frame grabber settings. Peak volumes were used for calculations.

Methanolic solutions corresponding to 4–16 μg of lovastatin or simvastatin (8–32 μL of 0.5 mg L^{-1} solution) per spot were applied on the plates to perform calibration. Calibration was performed 8-fold, each time with independent weighing and solvent dilution.

20 accurately weighed tablets were first ground in a mortar. The amount corresponding to 31.25 mg of the active substance was placed into 25 mL volumetric flask and shaken for 20 min with 10 mL of methanol. After shaking, the solutions were filtered and the amounts corresponding to 10 μg per spot were applied on the plates. For each kind of tablets, 7 independently weighed samples were analyzed and from each sample 6 spots were made in order to measure within-sample precision (error caused by spotting and measuring) and inter-sample precision (total error, including weighing and extraction errors).

RESULTS AND DISCUSSION

The chromatographic system used was chosen experimentally during comprehensive investigation of NP–TLC behavior of statins [10]. This system provides optimal R_f values of both drugs (0.44 for simvastatin and 0.47 for lovastatin), as well as optimum spot shape for quantitative measurements. Calibration densitograms are shown in Figure 1.

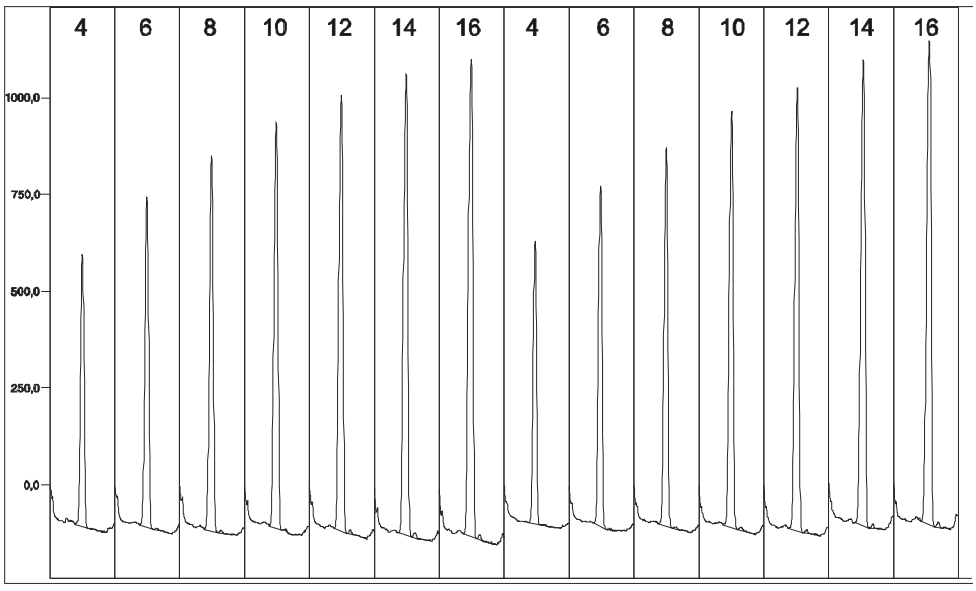

Figure 1. Sample densitograms of lovastatin obtained during calibration studies in the range 4–16 μg per spot (amounts labeled in the figure). The plate contains two calibration curves

4–16 μg per spot range used for calibration was relatively high due to the low UV absorbance of the investigated compounds [11]. Preliminary experiments have shown that at lower concentrations linear calibration plot was obtained as well but RSD values were unacceptable. At higher concentrations calibration plot was linear and precision was appropriate. 7 experimental points, each averaged from 8 replicate recordings, were used to prepare the calibration line in case of each drug and each analytical method. Peak area (densitometry) and peak volume (videodensitometry) were used for calculations.

LOD values calculated from the regression parameters of the calibration plot were: 0.85 μg and 0.74 μg per spot for densitometry of lovastatin and simvastatin, respectively, and 1.62 μg and 1.24 μg per spot for videodensitometry of lovastatin and simvastatin, respectively.

The results of calibration and determination were statistically evaluated. Statistical calculations were performed using R-project open source software [12]. Estimators of the calibration plots and their significances are presented in Table 1. The results of regression analysis are shown in Table 2.

Table 1. Estimators of calibration plots (linear: $y = a_1x + b_1$; quadratic: $y = a_2x + b2 + c_2x$), their standard errors and significance

	Estimate	SE	t	p
Lovastatin – densitometry				
a_1	179.02	5.07	35.2	$< 2e^{-16}$
a_2	351.27	18.03	19.4	$< 2e^{-16}$
b_1	1141.61	54.69	20.8	$< 2e^{-16}$
b_2	418.14	81.68	5.1	$< 2e^{-16}$
c_2	–8.61	0.88	–9.6	$< 2e^{-16}$
Simwastatin – densitometry				
a_1	178.26	3.57	49.8	$< 2e^{-16}$
a_2	297.40	13.10	22.7	$< 2e^{-16}$
b_1	1152.04	38.52	29.9	$< 2e^{-16}$
b_2	651.64	59.32	10.9	$< 2e^{-16}$
c_2	–5.95	0.64	–9.2	$< 2e^{-16}$

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Table 1. (Continuation)

	Estimate	SE	t	p
Lowastatin – videodensitometry				
a_1	322.90	13.52	23.8	$< 2e^{-16}$
a_2	602.66	95.01	6.3	$< 2e^{-16}$
b_1	1371.47	155.81	8.8	$< 2e^{-16}$
b_2	–18.86	489.73	–0.03	0.96
c_2	–12.71	4.28	–2.9	0.004
Simwastatin – videodensitometry				
b_1	1641.99	231.84	7.1	$< 2e^{-16}$
b_2	–1106.58	671.81	–1.6	0.10
a_1	523.68	20.12	26.0	$< 2e^{-16}$
a_2	1076.75	130.33	8.2	$< 2e^{-16}$
c_2	–25.13	5.87	–4.2	0.0001

Table 2. Results of calibration procedure – correlation coefficients, AIC values, lack-of-fit tests and Mandel's tests

	r	AIC	LOF	p(LOF)	Mandel's test	p(F)
Lovastatin – densitometry						
Linear	0.978	725.5	17.97	$4.35e^{-10}$	85.19	$1e^{-12}$
Quadratic	0.991	670.4	0.27	0.89		
Cubic	0.992	672.4	0.36	0.77		
Simvastatin – densitometry						
Linear	0.988	686.3	16.75	$1.26e^{-09}$	93.91	$2e^{-13}$
Quadratic	0.995	634.6	0.48	0.75		
Cubic	0.996	634.9	0.12	0.94		

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Table 2. (Continuation)

	r	AIC	LOF	p(LOF)	Mandel's test	p(F)
Lovastatin – videodensitometry						
Linear	0.965	693.9	2.30	0.07	8.82	0.0004
Quadratic	0.977	687.3	0.27	0.84		
Cubic	0.978	688.8	0.18	0.83		
Simvastatin – videodensitometry						
Linear	0.967	732.12	4.77	0.002	18.32	$9e^{-05}$
Quadratic	0.976	717.72	0.47	0.70		
Cubic	0.977	718.6	0.24	0.78		

Correlation coefficient R^2 is the most popular measure of the quality of linear and polynomial regression. R^2 value in linear calibration is, however, not very informative – it might be high in linear least squares method applied to significantly curvilinear data [13]. Thus, we have decided to perform more complex evaluation of the data to test linearity of calibration, similarly as it was done in case of fibrates in our previous work on hypolipidemic agents [14, 15].

All three plots were proven to be curvilinear applying common statistic tests: regression with marked residuals, the „lack-of-fit" test, and Mandel's fitting test (rejected hypothesis of linearity) was finally used to choose. The optimum polynomial order found by AIC (Akaike's Information Criterion) was 2 (quadratic equation).

Analysis of tablets was performed for 6 independently weighed samples. Each of them was applied onto a plate 7 times (42 results for each formulation). We have investigated 3 formulations available on the market to check the resistance of our method against different tablet matrices. The obtained sample densitogram is shown in Figure 2.

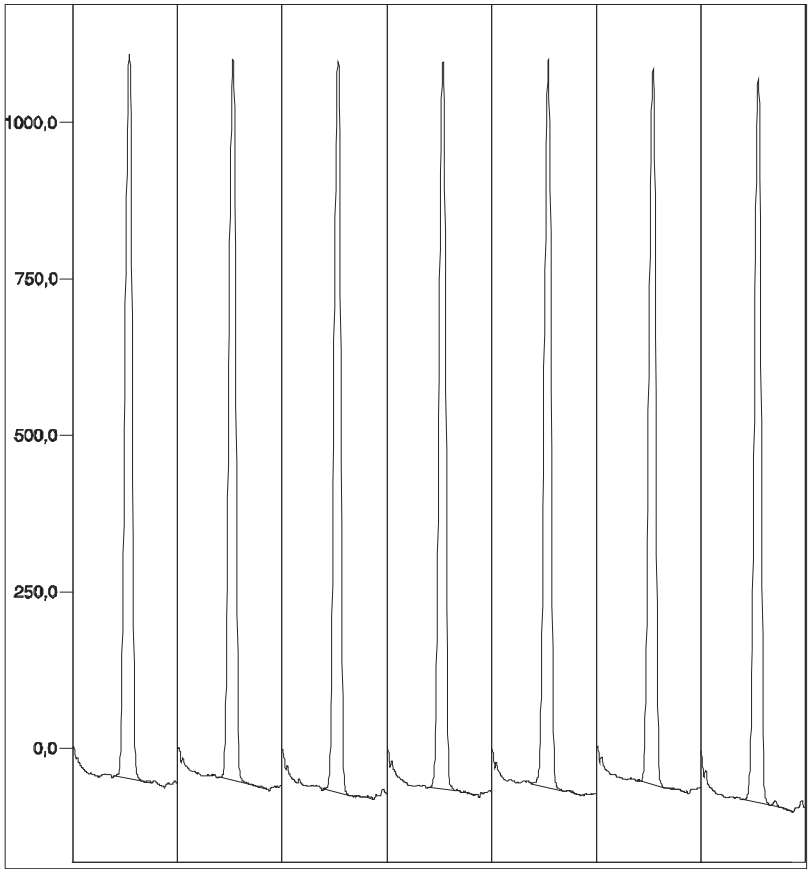

Figure 2. Sample densitograms obtained during analysis of tablets containing lovastatin. One plate was spotted 7-fold with one tablet sample

Videodensitometry was performed in independently prepared samples (densitometric plates were not used in videodensitometry to prevent autocorrelation of errors). The results gave the possibility to perform one-way ANOVA test and to estimate the contribution of sample preparation and spotting/measurement steps to the error of the method. The results are presented in Table 3. Recoveries are in an acceptable range (97.2–104.2% for densitometry and 96.7–106.2 for videodensitometry) and the results do not differ from the declared contents (checked with the t-test). Additionally (not shown in the table), in all cases the Shapiro-Wilk test did not detect any deviation from normal distribution. RSDs ranged from 1.43% (densitometry of Simvachol) to 8.88% (videodensitometry of Simvahexal) confirming acceptable precision.

Table 3. Results of determination of simvastatin and lovastatin in six commercially available pharmaceutical formulations

	Recovery	%RSD	t	p(t)	Preparation error	Sampling error
Densitometry						
Lovasterol	104.2	5.73	1.75	0.13	67.2	32.8
Liprox	97.9	2.88	–1.77	0.11	40.7	59.3
Lovastin	100.8	3.79	–2.00	0.10	46.0	54.0
Simvasterol	97.2	2.54	–2.59	0.06	32.5	67.5
Simvachol	101.7	1.43	2.58	0.06	15.5	84.5
Simvahexal	102.2	2.56	2.49	0.08	29.5	70.5
Videodensitometry						
Lovasterol	104.6	5.15	2.11	0.08	33.6	66.4
Liprox	95.8	2.49	–2.15	0.07	75.5	24.5
Lovastin	96.7	8.47	–0.79	0.46	14.9	85.1
Simvasterol	104.1	6.38	1.50	0.19	55.5	44.5
Simvachol	106.2	5.49	2.12	0.08	23.8	76.2
Simvahexal	96.8	8.88	–0.79	0.46	60.8	39.2

The elaborated methods were compared with respect to precision and accuracy (Tab. 4).

Table 4. Comparison of precision by Snedecor test (F, p(F)) and of accuracy by Student (t, p(t) and Wilcoxon (W, p(W)) tests

	F	p(F)	t	p(t)	W	p(W)
Simvasterol	7.19	0.049	–2.35	–0.054	6	0.064
Simvachol	15.90	0.008	–1.83	0.119	10	0.228
Simvahexal	14.37	0.010	1.92	0.105	28	0.132
Lovasterol	1.22	0.826	–0.11	0.910	15	0.688
Liprox	1.36	0.740	0.696	0.502	24	0.393
Lovastin	7.57	0.044	0.063	0.951	22	0.588

Precision and accuracy comparisons were performed applying F-Snedecor test, and either t-Student or Wilcoxon test, respectively. There was no difference in precision

between the methods in case of determination of Liprox and Lovasterol. In case of other drugs, densitometry was significantly more precise. Accuracy of both methods was equal – the results are homogenous.

CONCLUSION

HPTLC combined with densitometry or videodensitometry was found to be an accurate and precise alternative to HPLC methods for determination of simvastatin and lovastatin in tablets. Its advantages are: low cost of reagents, simplicity, satisfactory speed, and high efficiency. The proposed procedure shall be taken into consideration as a routine drug control method.

Acknowledgement

Donation of pure lovastatin and simvastatin samples by Polfa-Grodzisk Company is gratefully acknowledged.

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Received March 2007
Revised July 2007
Accepted September 2007