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Journal of Pharmaceutical and Biomedical Analysis
21 (2000) 1243–1248

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Second-derivative UV spectrometric determination of simvastatin in its tablet dosage form

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Accepted 27 September 1999

Abstract

Simvastatin, a highly effective cholesterol-lowering agent, has been widely used for the treatment of hypercholesterolemia. During the development of simvastatin solid dosage form, formulation compositions were constantly varied to define a suitable matrix. A fast and reliable method for the dissolution and release testing of simvastatin was highly desirable to support formulation screening. A second derivative UV spectroscopic method was developed for determination of simvastatin in the tablet dosage form. After carefully choosing a zero-crossing technique of second derivative UV measurement at 243 nm, the selectivity and sensitivity of simvastatin was comparable to the previously developed HPLC method. In comparison with the direct UV method, second derivative UV spectroscopy eliminates the interference from UV absorbing excipients such as ascorbic acid, which often results in a bias of 2–10%. This method is also fast and economical in comparison to the more time-consuming HPLC method regularly used for formulation screening. Finally, this method has been validated to be precise and accurate, and is demonstrated to be an excellent alternative to HPLC method for the dissolution and release testing of simvastatin in the solid dosage form.

Keywords: Simvastatin; Second derivative UV; Dissolution; Release testing

1. Introduction

Derivative UV spectroscopy has been widely used as a tool for quantitative analysis, characterization, and quality control in agricultural [1,2], pharmaceutical [3–6], and biomedical fields [7–12]. This technique offers various advantages over the conventional absorbency methods such as the discrimination of the sharp spectral features over the large bands and the enhancement of the resolution of overlapping spectra. As a result, derivative spectroscopy usually provides much better fingerprints than the traditional absorbency spectra. This outstanding feature coupled with zero crossing, least square deconvolution, or Fourier transform data processing technique has received increasing attention in single and multi-component quantitative analysis of pharmaceutical drug substances, especially in UV absorbing matrices [13]. For example, derivative UV spectroscopy has been used for the quantification of thiazide diuretics, acetaminophen and cyanocobalamin in the

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0731-7085/00/$ - see front matter
PII: S0731-7085(99)00231-9

presence of degradation products and preservatives [14–16].

Simvastatin, a highly effective cholesterol-lowering agent (Fig. 1), was developed as tablet dosage forms for the treatment of hypercholesterolemia. At initial formulation screening stage, formulation composition was constantly varied during a highly compressed time frame. A fast and reliable method for the dissolution and release testing of simvastatin was highly desirable. Objective of this study was to develop an alternative analytical method, to the more time consuming HPLC method, which can be used regularly and for formulation screening. A second derivative UV spectroscopy was developed to support formulation development of simvastatin in a sustained release solid dosage form.

2. Experimental

2.1. Materials

Simvastatin bulk drug was available from Merck Research Laboratories (Rahway, NJ). Simvastatin tablets and the placebo product were manufactured by Pharmaceutical Research and Development of Merck Research laboratories (West Point, PA) Acetonitrile, methanol, sodium dihydrogen phosphate were from Fisher Scientific (Fair Lawn, NJ) and used as received. Sodium dodecyl sulfate (SDS) was provided by Sigma Chemical Co. (St. Louis, MO) and used as received.

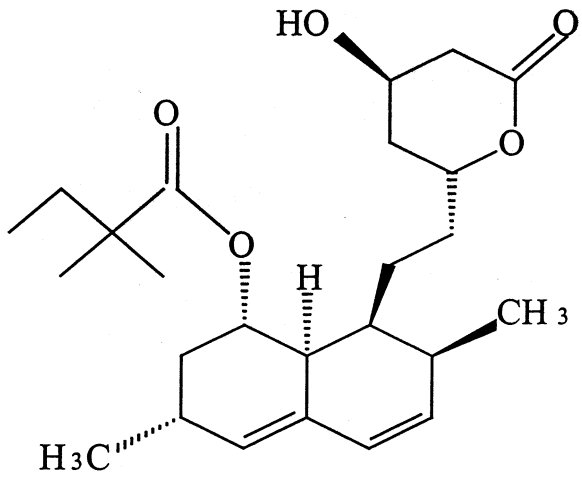

Fig. 1. Chemical structure of simvastatin.

2.2. Methods

2.2.1. Dissolution method conditions

Dissolution USP Apparatus 2 (paddle) with the paddle speed of 75 rpm was used. The tablets were inserted into a plastic coated helical sinker prior to dropping into the dissolution medium which consisted of 0.7% sodium dodecyl sulfate in 0.01 M sodium phosphate, pH 6.8 with a medium volume of 900 ml and temperature controlled at 37 ± 0.5°C.

The sample solutions were directly taken from the dissolution vessel through a syringe capped with a Vankel full flow 35 μm filter.

2.2.2. HPLC method for dissolution testing

A Hypersil ODS column (4.6 mm I.D. × 250 mm) at ambient temperature was eluted with a mobile phase consisting of acetonitrile/sodium phosphate buffer (0.025 M, pH 4)/methanol (55:33:12, v/v/v) at a flow rate of 2.0 ml/min. Simvastatin was determined by UV detection at 252 nm. The injection volume was 40 μl and run time was 5 min.

2.2.3. Sample solution preparation to content uniformity testing

For release testing, one tablet was placed into each of ten 100-ml volumetric flasks. The volume was diluted with a diluent consisting of acetonitrile/0.025 M phosphate buffer, pH 4.0 (65:35, v/v), and was stirred with a stirring bar for at least 3 h at fast speed until the tablets were dispersed in the solution. Samples were further diluted with 5 ml of the stock solution to 100 ml with the sample diluent consisting of acetonitrile/0.025 M phosphate buffer, pH 4.0 (65:35, v/v). An aliquot was centrifuged and the supernatant was analyzed.

2.2.4. HPLC method for content uniformity

A Hypersil ODS column (4.6 mm I.D. × 250 mm) thermostat at 45°C was eluted with a mobile

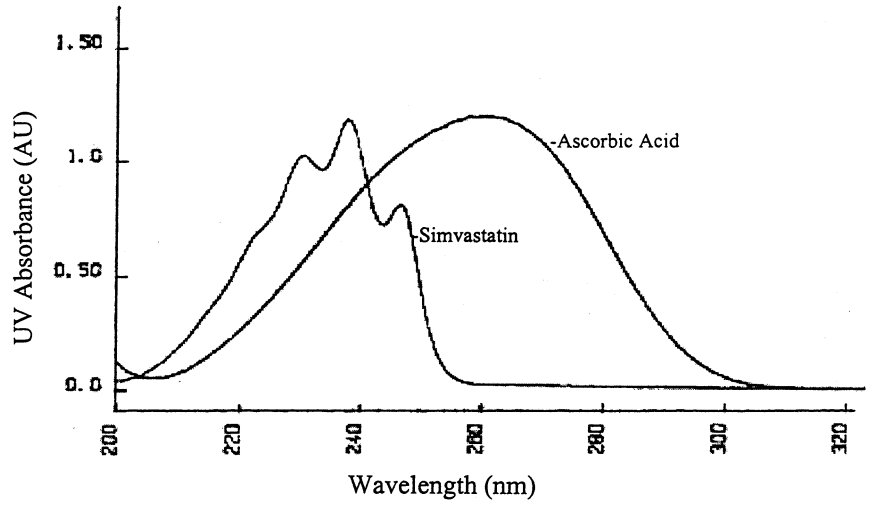

Fig. 2. UV spectra of simvastatin and ascorbic acid.

phase consisting of Acetonitrile/Sodium phosphate buffer (0.025 M, pH 4) (65:35, v/v) at a flow rate of 1.5 ml/min. Simvastatin was determined by UV detection at 238 nm. The injection volume of 50-μl and run time was 22 min.

2.2.5. Standard solution preparation

For release testing of simvastatin sustained release tablets, 20 mg of simvastatin reference standard was accurately weighed into a 100 ml volumetric flask and dissolved in the diluent consisting of acetonitrile/0.025 M phosphate buffer, pH 4.0 (65:35, v/v). The above stock solution (5-ml) was further diluted to 100 ml in a 100-ml volumetric flask to give the standard solution.

For dissolution testing, 36 mg of simvastatin reference standard was accurately weighed into a 200 ml volumetric flask and dissolved in no more than 10 ml methanol and diluted to 200 ml with a medium consisting of sodium dodecyl sulfate in 0.01 M phosphate buffer, pH 6.8. The above stock solution (25 ml) was further diluted to 200 ml in a 200-ml volumetric flask.

2.3. Apparatus

Spectrophotometric analyses were performed with a microcomputer-based Perkin–Elmer Lambda-6 UV-Visible double beam spectrophotometer equipped with an Epson Printer. It was interfaced with an Epson Equity III + Data Station (Q201A) via a standard RS232C interface for storage of spectra. The Epson printer (Model EX-800) was linked to the data station. Suitable settings are: slit width 2 nm, response time 5 s scan speed 60 nm/min and second derivative mode. A 10-mm silica cuvette suitable for the far-UV region was used in this study.

The HPLC analysis was performed on a Waters 600 liquid chromatograph with a 717 plus autosampler, 996-photodiode-array detector and a thermostat oven compartment. Measurements were made with a 50-μl-injection volume at 45°C; the detector wavelength was set at 238 nm. Routine analyses were carried out isocratically on a Hypersil 5 micron ODS, (25 cm × 4.6 mm) at a flow rate of 1.5 ml/min.

3. Results and discussion

With its diene chromophore, simvastatin exhibits an absorbency maximum at 238 nm as shown in Fig. 2. Determination of simvastatin in its tablet dosage form using direct UV measurement was attempted. However, under most circumstances, pronounced interference from other excipients particularly ascorbic acid was observed

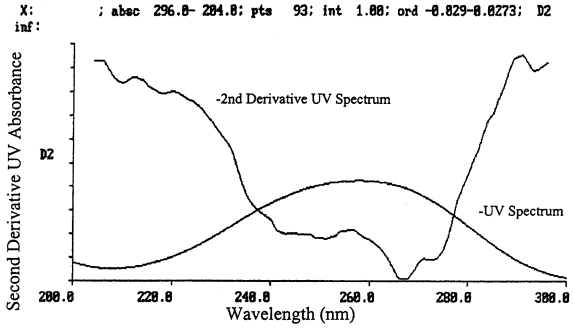

Fig. 3. UV and second derivative UV spectra of placebo tablet solution.

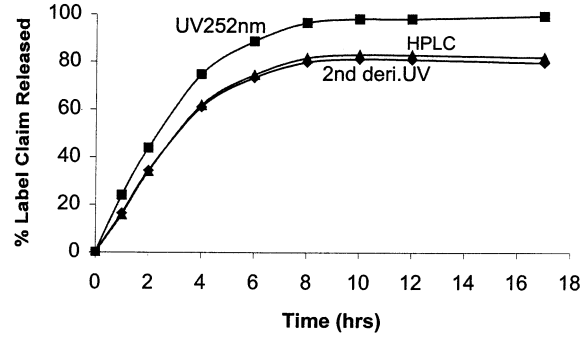

Fig. 5. Dissolution profiles of simvastatin developmental tablets obtained using (1) direct UV; (2) second derivative UV; and (3) HPLC.

which often give a bias of 2–10%. A typical UV spectrum of a placebo tablet is also shown in Fig. 3, which indicates a significant UV response with absorption maximum at 260 nm. Based upon the direct UV spectroscopic data, there is no wavelength where simvastatin can be accurately quantified without substantial background interference. However, the difference do exist between the second derivative UV spectra of simvastatin and the excipients in placebo tablets, which indicates the feasibility of a derivative UV method. The second derivative UV spectra of simvastatin and the excipients in placebo tablets were subsequently measured. As demonstrated in Fig. 3 and Fig. 4, simvastatin can be measured at 243 nm with little interference in the second derivative mode.

The dissolution testing was conducted on simvastatin tablet dosage form and the sample solutions were analyzed using direct and second derivative UV spectroscopy followed by currently used HPLC method. With second derivative UV spectroscopic method, quantification of simvastatin was achieved by measurement of the peak-to through height of the signal corresponding to the second derivative of the direct spectrum at 243 nm. As indicated in Fig. 5, second derivative UV method gives highly comparable results to HPLC method. As expected, the accuracy of direct UV spectroscopic method suffers from substantial matrix interference.

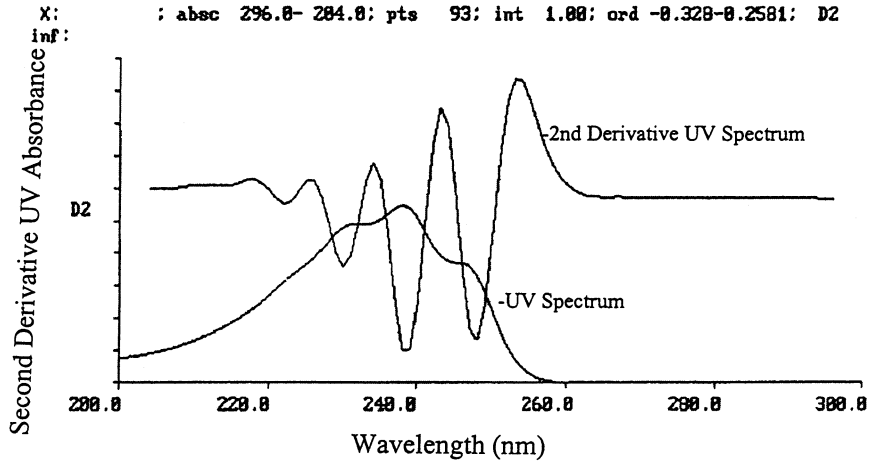

Fig. 4. UV and second derivative UV spectrum of simvastatin.

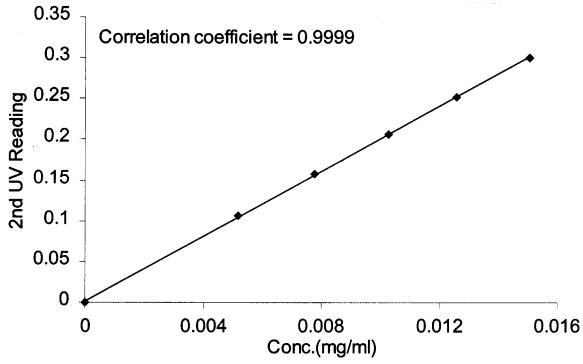

Fig. 6. Linearity of second derivative UV absorbance verus simvastatin concentrations ranging from 50 to 150% of Simvastatin method concentration (0.01 mg/ml).

Table 1
Accuracy of second derivative method determined by the recovery of simvastatin from placebo tablets spiked with simvastatin solution

Level (%)	mg Added	mg Recovered	% Recovery
50	10.79	10.85	100.5
50	9.97	10.13	101.6
75	15.69	15.82	100.8
75	15.38	15.27	99.3
100	20.74	20.72	99.9
100	20.35	2015	99.0
125	24.92	24.67	99.0
125	25.31	24.98	98.7
150	29.88	29.33	98.1
150	30.34	30.04	99.0
Average			99.6
RSD (%)			1.1

Table 2
Measurement precision

Injection	2nd UV reading	Injection	2nd UV reading
1	0.2123	6	0.2079
2	0.083	7	0.2077
3	0.2076	8	0.2072
4	0.2068	9	0.2078
5	0.2083	10	0.2077
Average			0.2087
RSD (%)			1.0

3.1. Method validation

Linearity of second derivative spectra of simvastatin concentration was established by preparing one series of simvastatin solution ranging from 5 to 15 μg/ml which corresponds to 50–150% of method concentration (0.01 mg/ml). The second derivative spectra were recorded using the diluent as a blank. All solutions were measured for absorbency from 200 to 300 nm. Using regression analysis the following equation was obtained for simvastatin:

$$y = 19.927x + 0.0014 (r^2 = 0.9999)$$

where y is the absolute value of the second derivative of simvastatin absorbency at 243 nm and x is the concentration of simvastatin (mg/ml) (Fig. 6).

The accuracy of the method was determined by investigating the recovery of the simvastatin at five levels ranging from 50 to 150% of the method concentration (0.01 mg/ml) from solution-spiked placebo. The results are shown in Table 1, which indicate excellent recoveries ranging from 98.1 to 101.6% with a mean of 99.6% (RSD = 1.1%, N = 10).

The measurement precision was determined by performing ten replicate injections of standard solutions at the method concentration (0.01 mg/ml). The RSD was found to be 0.73% by second derivative absorbency measurement (Table 2).

The method precision for sample was determined by the analysis of ten simvastatin tablets. For quantification of simvastatin, the sample solutions were bracketed with external standard solutions. In addition, both HPLC and direct UV method analyzed the same tablet solution. The results shown in Table 3 demonstrate that data generated by second derivative UV method agree well with HPLC results. In comparison with the data generated by second derivative UV, direct UV measurement have a bias of 5–10%, further indicating background interference.

4. Conclusion

A reliable second derivative UV spectroscopic method was developed for the analysis of simvas-

Table 3
Assay results for ten simvastatin developmental tablet solutions by UV, second derivative UV and HPLC methods

Sample (tablet no.)	% Claim (UV)	% Claim (HPLC)	% Claim (2nd UV)
1	101.0	94.7	94.8
2	100.9	93.8	93.7
3	101.0	94.3	94.7
4	101.7	95.6	94.2
5	101.2	94.9	95.8
6	101.1	94.8	95.3
7	101.8	94.6	96.7
8	101.6	93.9	96.4
9	98.3	93.0	90.9
10	99.2	94.0	93.8
Average	100.8	94.4	94.6
RSD (%)	1.1	0.8	1.7

tatin in its developmental tablet dosage form. Two major features of this technique were observed during this study: (1) it is very efficient and offers high sample throughput by comparison with HPLC methods. Therefore, it undoubtfully renders in-time data turnaround during formulation development, and (2) it offers comparable accuracy by eliminating the interference of formulation excipients such as ascorbic acid; unlike direct UV spectrometric method which often gave 2–10% bias, resulted from matrix interference. Finally, this method can be used as an excellent alternative to HPLC for formulation screening, release and dissolution testing of simvastatin in the solid dosage form.

Acknowledgements

The authors would like to thank Michael McGlynn for provide simvastatin tablets. Also the authors thank reviewers of Merck for their valuable suggestions.

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