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Feasibility of lovastatin analysis by packed column supercritical fluid chromatography with ultraviolet detection

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Abstract

A reliable supercritical fluid chromatography (SFC) method was developed for the analysis of lovastatin, a hypocholesterolaemic drug, from MEVACOR®. Methanol-modified carbon dioxide was shown to elute the drug, and its dehydrolovastatin and hydroxy acid lovastatin degradation products from a Hypersil® silica column. However, the hydroxy acid lovastatin was found to tail in this mobile phase. The phenomena was eliminated by the addition of trifluoroacetic acid [Haouck, S. Thomas, D. K. Ellison, Talanta 40 (1993) 491] to the mobile phase which permitted the drug and its two main degradation products to all elute from the Hypersil® silica column in under 6 min with symmetrical peak shape. Chromatographic limit of detection (LOD) and limit of quantification (LOQ), linear dynamic range (LDR), and injection precision were obtained in order to assess the chromatographic performance of the SFC system for the lovastatin separation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Lovastatin analysis; Supercritical fluid chromatography; Ultraviolet detection

1. Introduction

The analysis of drugs and their potential degradates is very important to control the potency, purity, safety, and efficacy of pharmaceutical products. Currently, most stability indicating assay methods in the pharmaceutical field utilize reversed phase high performance liquid chromatography (HPLC) coupled with ultraviolet detection. As in the case of lovastatin (Fig. 1), a hypocholesterolemic drug, HPLC/UV is often preferred due to the polar nature of the drug [1–4]. A method involving derivatization with 4-nitrobenzoic acid to improve ultraviolet detection of lovastatin and isocratic reversed phase HPLC elution has been reported for monitoring lovastatin in fermentation broth [1]. With improvements in detector sensitivity, the derivatization step has become unnecessary for analysis of

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lovatatin [2–4]. For example, the analysis of lovastatin in rat and dog bile and plasma by a gradient elution method has been reported [2]. Unfortunately, HPLC is an unattractive methodology because it produces large volumes of solvent waste that are becoming increasingly expensive to dispose. One alternative to HPLC is packed-column supercritical fluid chromatography (SFC) with a carbon dioxide based mobile phase which yields greater resolution per unit time and generates only a small fraction of the organic solvent waste.

In this study, a rapid and selective SFC method which utilizes UV detection developed to demonstrate the feasibility of analysis of lovastatin and the degradation products hydroxy acid lovastatin and dehydrolovastatin (Fig. 1) will be decribed. Under optimized chromatographic conditions, detector performance was assessed for the SFC-UV system. The acuracy, precision, and analysis time of the procedure will be discussed.

2. Experimental

2.1. Instrumentation

A prototype of the Hewlett Packard model G1205 SFC system (Little Falls, DE) was used for the chromatographic separations. System pressure was maintained electronically by a computer-controlled, back pressure regulator which allowed the flow rate and pressure to be independently controlled. The mobile phase flow rate was measured as a liquid at the pump. Organic modifier was introduced via an auxillary pump. An internal 5 μl loop injector was used to introduce sample to the column. A standard Hewlett Packard model 1050 multi-wavelength detector (MWD) was utilized for detection which employed a 13 μl high pressure flow cell (10 mm path length). A 250 × 4.6 mm Hypersil® silica (dp = 3 μm) column (Keystone Scientfic Inc., Bellefonte, PA) was used.

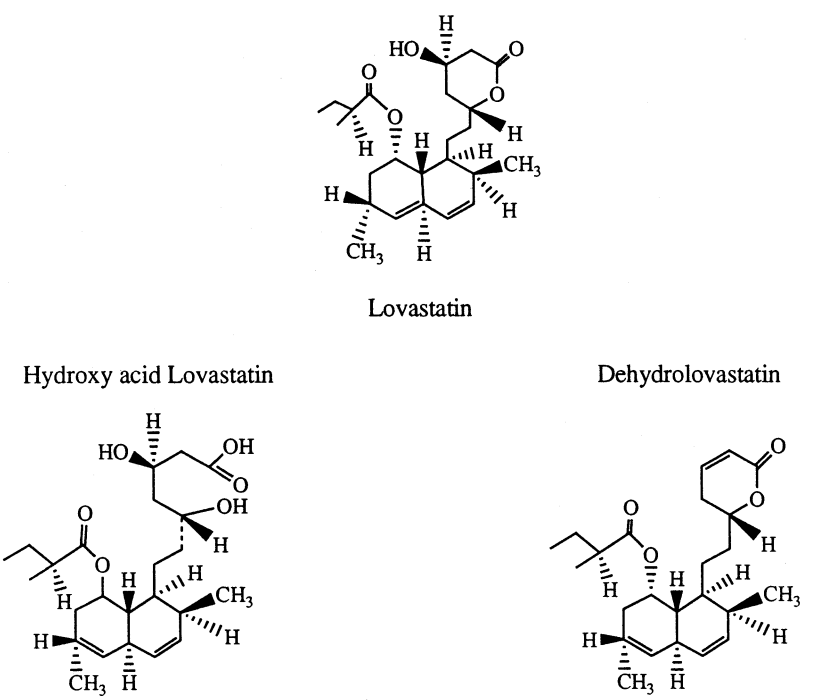

Fig. 1. Chemical structure of lovastatin and its two main degradates.

Table 1
Effect of modifier concentration on SFC analysis of lovastatin[a]

	Modifier concentration (vol.%)					
	6	8	10	12	14	HPLC[b]
Retention time (min)	8.37	5.32	4.05	3.34	2.89	8.12
Retention time RSD	1.5	1.2	1.4	0.3	0.9	0.3
t_0 (min)	1.54	1.51	1.53	1.55	1.54	1.60
k'	4.58	2.55	1.70	1.23	0.93	4.07
$w_{1/2}$ (min)	0.215	0.128	0.090	0.071	0.060	0.198
N	8033	9609	11367	12271	12718	6100
h (mm per plate)	0.031	0.026	0.022	0.020	0.002	0.041
t_f	1.03	1.11	1.18	1.26	1.34	1.30
Peak area RSD	0.5	0.3	3.0	3.0	0.3	2.0

[a] SFC analysis conditions were as follows: 25 cm × 4.6 mm i.d. Hypersil® Si column; temperature, 45°C; pressure, 200 bar; flow rate, 2.0 ml/min; injection volume, 5 μl. Lovastatin concentration was 1 mg/ml dissolved in methanol. All peak parameters were calculated based upon five replicate injections of a lovastatin standard.
[b] Conditions are described in Section 2.

2.2. Chemicals

Methanol, acetonitrile, and water were of high purity (HPLC grade, Fisher Scientific Co., Fair Lawn, NJ). The carbon dioxide was SFE/SFC grade (Air Products and Chemicals, Inc., Allentown, PA) with no helium head. The lovastatin and MEVACOR® were provided by Merck Research Laboratories (West Point, PA).

2.3. HPLC-UV assay

Solutions of lovastatin were assayed by HPLC using a HP 1050 isocratic HPLC pump connected to a Valco model EQ-60 LC injector, a HP 1050 variable wavelength UV detector, and a HP 3396 integrator. A 4.6 × 250 mm Hypersil® octadecyl derivatized silica column from Keystone Scientific (Bellefonte, PA) was utilized. A flow rate of 1.5 ml/min, an injection volume of 10 μl, and a UV detection of 230 nm were also utilized. The isocratic mobile phase was composed of 55% acetonitrile, 12% methanol, and 33% water with the pH adjusted to 4 using a phosphate buffer. The lovastatin solutions were obtained by liquid-solid extraction at room temperature of MEVACOR® into a mixture of acetonitrile/water.

3. Results and discussion

The goal of this work was to develop a rapid efficient SFC separation of lovastatin and its degradation products with similar precision and accuracy as the currently used HPLC assay. In addition, the reduction of solvent waste by the SFC method was also pursued. Isocratic, isothermal, and isobaric SFC conditions were utilized to ensure maximum accuracy and precision. To obtain high solvating strength of the sub/supercritical fluid, a low oven temperature (45°C) and moderate pressure (200 bar) were used. A liquid flow rate of 2.0 ml/min was utilized with the 4.6 mm i.d. packed column. Although the lovastatin from a fermentation broth was found to be soluble in 100% CO_2 by Larson and King [5], the addition of methanol modifier to the mobile phase was required in this work to elute the drug from a silica column.

In an attempt to further understand the effects of methanol modifier on the SFC analysis of lovastatin, peak parameters [capacity factor (k'), peak width at half height ($w_{1/2}$), plates/column (N), plate height (h), and peak tailing factor (t_f)] were calculated (Table 1). Peak parameters were calculated for the corresponding optimized HPLC lovastatin analysis as well and they are provided

for comparison in Table 1. All values were based on five replicate injections of a single solution. From Table 1, both the plate height, capacity factor, and peak width at half height were significantly affected by the modifier concentration in the isocratic SFC experiments. Specifically, the plate height was reduced with increasing modifier which meant the separation was more efficient at the higher modifier concentrations. Similarly, the capacity factor was lowered with increasing modifier which corresponded to faster analysis times. The faster analysis times were expected since the higher modifier concentration would increase the mobile phase solvating strength. A modifier concentration of 10% (v/v) was chosen for further separations since it represented the best compromise between analysis time, column efficiency, resolution, and peak shape.

The effect of CO_2 pressure on the chromatographic peak parameters was also examined (Table 2). Capacity factor was the only parameter to significantly change in a regular manner with an increase in pressure. This was thought to be caused by higher solvating strengths of the supercritical fluid at higher density values. Plate height, plate number, and peak shape were essentially unchanged. A CO_2 pressure of 230 bar was chosen as the best compromise between column efficiency, peak shape, and speed of analysis.

After determining the best initial parameters for the analysis of lovastatin, a separation involving lovastatin and the two main degradation products, hydroxy acid lovastatin and dehydrolovastatin was attempted. The degradation products were produced by adding acid (100 μl of trifluoroacetic acid) to a 1000 ppm solution of lovastatin at room temperature dissolved in acetonitrile/water (80:20). Since a small injection volume (5 μl) was used, the presence of water which was introduced during sample preparation did not affect the SFC method. At 10% (v/v) methanol-modifier, the hydroxy acid lovastatin peak eluted with the solvent peak, while, lovastatin and dehydrolavastatin were baseline resolved. In an effort to retain the hydroxy acid on column, the modifier concentration was reduced to 6% (v/v) (Fig. 2A). From the chromatogram, it can be seen that the hydroxy acid lovastatin peak moved away from the solvent peak but the peak tailed. To reduce the peak tailing, an acidic additive (0.5% (v/v) trifluoroacetic acid) was added to the methanol modifer. Consequently, a typical packed-column SFC/MWD separation of lovastatin (peak 3) from hydroxy acid lovastatin (peak 1) and dehydrolovastatin (peak 2) is shown in Fig. 2B using 6% (v/v) trifluoroacetic acid-methanol (0.5:99.5) modified CO_2. Note that the retention order for dehydrolovastatin and lovastatin has reversed in going from reversed phase HPLC (Fig. 2C) to SFC (Fig. 2B). Whereas, the apparently more polar hydroxy acid derivative elutes earliest in both HPLC and SFC cases. Retention in SFC is more difficult to predict as both solute interaction with mobile phase and stationary phase, solute volatility, and solubility in the mobile phase

Table 2
Effect of CO_2 pressure on SFC analysis of lovastatin[a]

	CO_2 pressure (bar)			
	200	230	260	290
Retention time (min)	4.02	3.87	3.73	3.64
t_0 (min)	1.52	1.51	1.53	1.51
k'	1.67	1.58	1.48	1.42
$w_{1/2}$ (min)	0.090	0.087	0.085	0.084
N	10 900	10 800	10 800	10 400
h (mm per plate)	0.023	0.023	0.023	0.024
t_f	1.18	1.17	1.16	1.16

[a] SFC analysis conditions were as follows: 25 cm × 4.6 mm i.d. Hypersil® Si column; temperature, 45°C; mobile phase, 10% (v/v) methanol-modified CO_2; flow rate, 2.0 ml/min; injection volume, 5 μl. Lovastatin concentration was 1 mg/ml dissolved in methanol.

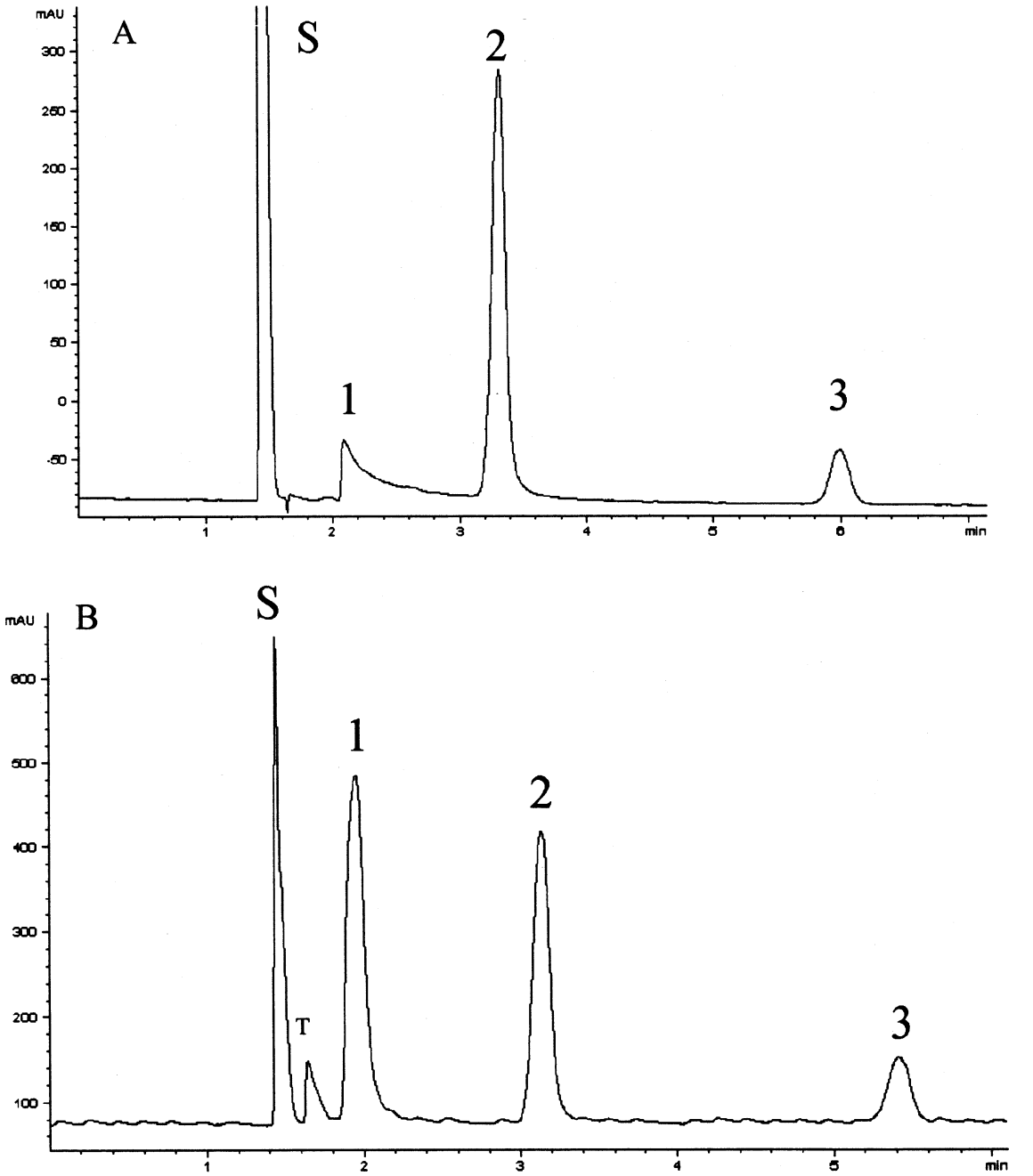

Fig. 2. SFC/MWD lovastatin separation. SFC conditions: pressure, 230 bar; 25 cm × 4.6 mm i.d. Hypersil® Si column; temperature, 45°C; flow rate, 2.0 ml/min; injection volume, 5 μl. (A) mobile phase, 6% (v/v) methanol-modified CO_2; (B) mobile phase, 6% (v/v) trifluoroacetic acid–methanol (0.5–99.5) modified CO_2; (C) HPLC-UV conditions listed in Section 2. Peak identity was: (S) injection solvent (acetonitrile–water (80:20) with trifluoroacetic acid), (T) trifluoroacetic acid, (1) hydroxy acid lovastatin, (2) dehydrolovastatin, and (3) lovastatin.

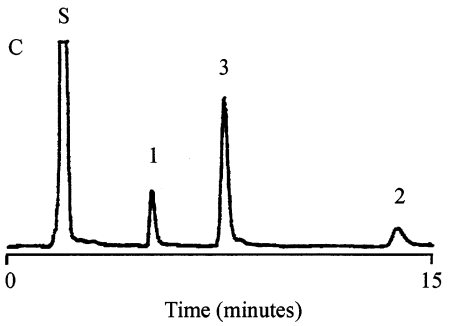

Fig. 2. (*Continued*)

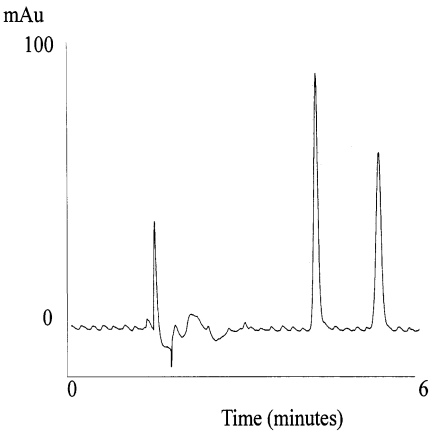

Fig. 3. SFC-MWD analysis of lovastatin from Mevacor® tablets. Conditions listed in Fig. 2B. Peak identify as follows: (S) injection solvent, (H) 17-a-hydroxyprogesterone internal standard, and (3) lovastatin.

are influential. While SFC is generally considered to be normal phase chromatography, these data suggested a mixed retention mode. Because of the change in retention order, one could possibly use SFC and RP-HPLC as complementary techniques to confirm the identify of unknowns similar to normal and reversed phase HPLC.

3.1. SFC/MWD detector performance

Chromatographic limit of detection (LOD) and limit of quantitation (LOQ) for SFC coupled with a multiwavelength detector were calculated using the propagation of error method [6]. All pertinent calibration curve data are listed in Table 3. The linear dynamic range for the SFC/MWD system was determined to be two orders of magnitude (12–3600 μg/ml lovastatin dissolved in methanol) at 230 nm ($r = 0.9996$). Injection reproducibility based upon peak areas for SFC/MWD was found to be 1–3% RSD for replicate injections (5 μl, $n = 5$) of individual samples at the previously stated lovastatin concentrations. The day-to-day injection precision was estimated to be 2.2% measured over 3 days. A larger injection volume no doubt would have reduced the RSD value. The LOD of lovastatin was calculated to be 15 ng/μl or 15 ppm (e.g. 75 ng), and the LOQ was determined to be 50 ng/μl or 50 ppm (e.g. 250 ng) for a 5 μl injection loop. For the HPLC analysis, the LOD was found to be 20 ng/μl or 20 ppm (e.g. 200 ng) with a 10 μl injection loop (RSD = 2.0%).

3.2. SFC versus HPLC analysis of lovastatin tablet

In order to directly compare both packed column SFC and HPLC, 10 mg potency MEVACOR® tablets were analyzed. The tablets ($n = 3$) were extracted into an acetonitrile/water (1/1) mixture at room temperature. An aliquot of each tablet extract was then assayed by both SFC/MWD and HPLC/UV. For SFC analysis, a 5 μl injection was employed; while, a 20 μl injection was used for the HPLC analysis. Since 5 μl is a relatively small injection volume, the acetonitrile/water extracts were directly injected into the SFC column even though the solution contained some water. SFC peak shape was found to be satisfactory under these conditions (Fig. 3) for reliable peak integration. The percent claim for the three tablet extracts is shown in Table 4. Lovastatin recoveries ($n = 3$) were 100% claim via HPLC/UV analysis and 97.7% claim via SFC/MWD. Statistical analysis via t-test on the results revealed that the HPLC/UV and SFC/MWD methods were equivalent.

While the SFC and HPLC methods yielded similar accuracy and precision, the HPLC method

Table 3
SFC/MWD calibration curve results[a]

	SFC/MWD (230 nm)
m[b]	5.03
I[b]	53.6
r[b]	0.9996
s_m[b]	0.0762
s_i[b]	277
$s_{y/x}$[b]	282
LOD (ppm)	15
LOQ (ppm)	50

[a] SFC analysis conditions were as follows: mobile phase, 6% (v/v) trifluoroacetic acid–methanol (0.5–99.5) modified CO_2; pressure, 230 bar; 25 cm × 4.6 mm i.d. Hypersil® Si column; temperature, 45°C; flow rate, 2.0 ml/min; injection volume, 5 μl.

[b] m is the slope, I is the intercept, r is the correlation coefficient, s_m is the error in the slope, s_i is the error in the intercept, $s_{y/x}$ is the point error.

generated more disposable waste than the SFC method (Table 5) and resulted in the analysis of fewer samples per hour. Since the number of samples per hour handled by the SFC method was six, more mobile phase was used by SFC than HPLC but since the mobile phase was only 6% solvent modified-CO_2 only 7.2 ml (out of 120 ml total) of disposable waste was generated in separating the six samples. The remaining 112.8 ml of mobile phase was CO_2 which could be vented to the hood. In the HPLC case only four samples could be analyzed per hour which gave rise to 90 ml of mixed mobile phase, all of which had to be disposed. Therefore, the SFC/MWD method appears to be far superior to the more established HPLC/UV method in that the SFC approach can perform 50% more samples per hour than the HPLC approach while generating only 7.2 ml of disposable waste compared to 90 ml of disposable waste via HPLC (i.e. solvent reduction over 90%).

Table 4
Comparison of SFC/MWD vs HPLC/UV for tablet analysis after liquid-solid extraction[a]

	SFC/MWD (percent of claim)	HPLC/UV (percent of claim)
Tablet 1	97.7	103
Tablet 2	96.0	97.8
Tablet 3	98.4	99.5
Average	97.8	100
RSD	1.6	2.7

[a] SFC/MWD conditions are as in Fig. 2B. HPLC/UV conditions are given in Section 2.

In summary, the SFC method was shown to be specific and/or reproducible for the separation of lovastatin from its two main degradation products. While the efficiency of the HPLC and SFC methods were comparable, the speed of the SFC separation (< 5 min) was found to be better than the isocratic reversed phase method. Both methods gave comparable resolution, limit of detection, and limit of quantification.

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