CHAPTER III

INVESTIGATION OF PRIMARY AND SECONDARY MODIFIERS FOR THE SUBCRITICAL EXTRACTION OF LOVASTATIN FROM MEVACOR® TABLETS WITH CARBON DIOXIDE

3.1 INTRODUCTION

Past studies in both super/subcritical fluid extraction (SFE) and chromatography (SFC) have primarily utilized carbon dioxide as the supercritical fluid (SF) because it is relatively inert, highly pure, nontoxic, exhibits readily attainable critical parameters (T_c=31 °C, P_c=71 atm), and has solvent power equivalent to common organic solvents such as hexane. Super/subcritical carbon dioxide has been proven to be an efficient medium in the extraction of non-polar and moderately polar compounds; however, its solvating power may be insufficient for the extraction of highly polar compounds such as most pharmaceuticals. As a result, the pharmaceutical industry has been reluctant to accept CO_2 as an extraction fluid. This limitation may be overcome by either using a more polar SF like ammonia, or by adding small amounts of polar organic solvents (i.e. modifiers) to carbon dioxide. A polar SF such as ammonia is rarely used due to its toxicity, reactivity, and extreme critical parameters (T_c=132 °C, P_c=111 atm). As a result, most pharmaceutical applications have utilized modified carbon dioxide. [1-4]

Modifiers generally serve two functions: a) increase the solvating power of the SF and b) facilitate the disruption of analyte-matrix interactions. [5] For instance, felodipine, an antihypertensive basic drug, was found to be soluble in 100% CO_2; however, when

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[1] *W.N. Moore, L.T. Taylor*, Anal. Chem., **67** (1995) 2030.
[2] *N.N. Dulta, A.P. Baruah, P. Phukan*, CEW, **26** (1991) 25.
[3] *A.L. Howard, M. Shah, P.I. Dominic, M.A. Brooks, J.T.B Strode, L.T. Taylor*, J. Pharm. Sci., **83** (1994) 1537.
[4] *K.A. Larson, M.L. King*, Biotech. Prog., **2** (1986) 73.
[5] *J.M. Levy, L. Dolata, R.M. Ravery, E. Storozynsky, K.A. Holowczak*, J. High Resolut. Chromatogr., **16** (1993) 368.

extracting a sustained-release tablet containing felodipine, only 60% was recovered with pure carbon dioxide under similar conditions. [3] To achieve quantitative extractions (97-103%), 8% (v/v) methanol-modified CO_2 was needed apparently to disrupt analyte/matrix interactions. Alternatively, the modifier may be more effectively used by introducing it directly to the matrix prior to extraction with CO_2.

The addition of a modifier to either the SF or to the matrix prior to SFE may not be sufficient for the extraction of multifunctional, highly polar, and ionic compounds. A secondary modifier (i.e. additive) may be added to the primary modifier to achieve successful analyte extraction or separation. The additives typically consist of relatively strong organic acids or bases and are usually added directly to the primary modifier (0.1%-5% (v/v)) rather than to the fluid or matrix. [6] The general guideline for the use of additives in SFC and SFE is that acidic analytes require acidic additives and basic analytes require basic additives. Additives have been used recently to improve peak shape and enhance separation in supercritical fluid chromatography of polar compounds. [6-8] Berger and Deye have demonstrated that compounds containing more than two carboxylic acid groups on a benzene ring could not be eluted from a sulphonic acid column with less than 20% methanol-modified CO_2; however when an additive such as citric acid was added to methanol-modified CO_2, benzene mono-, di-, and tricarboxylic acids could be separated and eluted. [9] In this case, the citric acid was said to cover active sites (exposed silanols) on the stationary phase surface, thus retention of the highly polar carboxylic acids was greatly reduced. Additives have also found some use in SFE, specifically in the extraction of basic polar compounds. A mebeverine alcohol metabolite was successfully isolated from dog plasma onto a SPE cartridge and extracted from the cartridge with SFE using methanol-modified CO_2 and isopropylamine. [10] Also, cocaine from human hair was

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[6] *T.A. Berger, J.F. Deye*, J. Chromatogr. Sci., **29** (1991) 26.
[7] *T.A. Berger, J.F. Deye*, ACS Symp. Ser., **488** (1992) 132.
[8] *T.A. Berger, W.H. Wilson*, J. Chromatogr. Sci., **31** (1993) 127.
[9] *T.A. Berger, J.F. Deye*, J. Chromatogr. Sci., **29** (1991) 141.
[10] *H. Liu, L.M. Cooper, D.E. Raynie, J.D. Pinkston, K.R. Wehmeyer*, Anal. Chem., **64** (1992) 802.

extracted using CO_2 modified with water and triethylamine. [11] In both cases, the basic additive served as an ion-suppresser thus favoring the extraction of the free bases. Therefore it was concluded that additives serve two main purposes including acting as ion-suppressers as well as reducing the activity of the stationary phase or matrix by covering active sites.

Although the solubility of an analyte may be high in the fluid, successful extraction of the analyte from a complicated matrix such as a tablet may be problematic due to large analyte/matrix interactions. It was, therefore, the objective of this study was to investigate the role of secondary modifiers (i.e. additives) for the extraction of a commonly used pharmaceutical compound from a complicated matrix such as a tablet. In this study, the analyte being extracted was neutral and contained no ionizable functionalities. Although it was expected that ion-suppression would not play a role in this study, it was of interest to examine the effect of additive type (acidic, basic, neutral) on its ability to cover active matrix sites and thus displace the polar analyte from the complicated tablet matrix.

The study was divided into four phases utilizing lovastatin, an antihypercholesterolemic drug, (active ingredient in MEVACOR® tablets) as the prototype drug. Phase A determined the effect of methanol on the extractability of lovastatin from an in-house prepared tablet mixture. The role of additive type (acidic, basic, and neutral) was then investigated in Phase B. The effect of additive and modifier concentration was investigated in Phase C. Once an optimum additive concentration was chosen (Phase D), the usefulness of the additive with methanol versus methanol-modified CO_2 alone in terms of overall extraction recovery and time needed to extract lovastatin directly from commercially available MEVACOR® tablets was examined. Finally, the reproducibility of the optimum SFE method was demonstrated.

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[11] *J.F. Morrison, W.A. MacCrehan,* Presented at the 5th International Symposium on SFE/SFC, January, 1994.

3.2 EXPERIMENTAL

All extractions were performed on the Isco Suprex Prepmaster (Lincoln, NE). Carbon dioxide (SFE/SFC grade) with helium headspace was donated by Air Products and Chemicals, Inc. (Allentown, PA). For all tablet extractions, either approximately 100 mg of in-house prepared tablet powder (Phases A-C) containing 10 mg of lovastatin (Merck Research Laboratories, West Point, PA) or a crushed MEVACOR® tablet (Phase D) containing 10 mg lovastatin was placed into an extraction vessel (5 mL volume (Phase A-B) or 3.5 mL volume (Phase C-D), Keystone Scientific, Bellefonte, PA) containing cotton balls. Cotton balls were used to reduce the dead volume of the vessel. The extraction and trapping conditions for this study are found in **Table 3.1.**

Extract Analysis

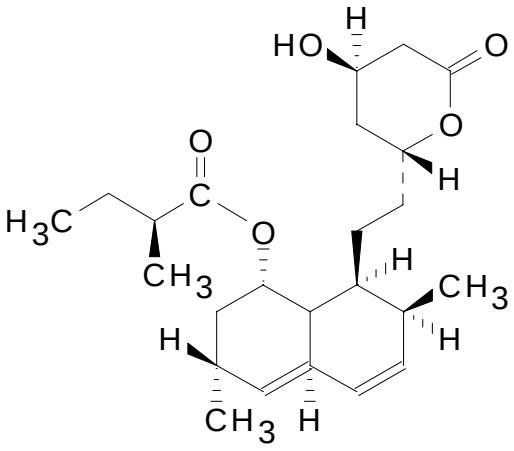
After the extraction, trapping, and recovery steps, approximately 0.4 mg of 17-α-hydroxyprogesterone (**Figure 3.1**) was added to the combined liquid trap/solid-phase trap rinses as an internal standard. The purpose of adding internal standard to the trap rinse and tandem liquid trap was to ensure good quantitation in case there were variations in solid-phase trap rinse volumes as well as evaporation losses in the tandem liquid trap during the extraction. A portion of each solution was then transferred to SFC vials for analysis.

A prototype of the Hewlett Packard Model G1205 SFC system (Little Falls, DE) was used for Phase A, B, and D (15% methanol-modified CO_2 mixtures and reproducibility) analyses. All other analyses were performed on the Gilson SF3™ SFC system (Middleton, WI). All separations were performed isocratically with a Hypersil®

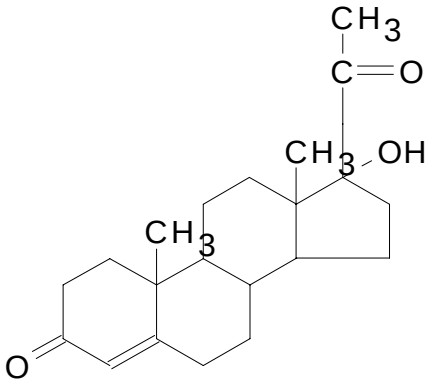
Table 3.1. Extraction and Trapping Conditions Used for Phases A-D

CO_2 Pressure	400 atm
Oven Temperature	40 °C
Liquid Flow Rate	2.0 mL/min.
Restrictor Temperature	50 °C
Solid Phase Trap	50/50 (w/w) Porapak Q/Glass Beads
Liquid Tandem Trap	Methanol
Liquid Tandem Trap Volume	5 mL, room temperature, 7 mL, room temperature (Phase D, reproducibility)
Collection Temperature (solid-phase)	40 °C
Desorption Temperature (solid-phase)	40 °C
Solid-Phase Trap Rinse Solvent/Volume	Methanol, 2.0 mL* (Phases A-D) Methanol, 5.0 mL (Phase D, reproducibility)
Rinsing Flow Rate	1.0 - 2.0 mL/min. (Phase D (reproducibility)

*Solid-phase trap was rinsed directly into liquid tandem trap following each dynamic extraction step when constructing extraction profiles.



(a)



(b)

Figure 3.1. Chemical Structures for Lovastatin (a) and 17α-Hydroxyprogesterone (b)

silica (4.6 mm i.d. X 25 cm, 3 μm particle diameter) column (Keystone Scientific, Bellefonte, PA) and a mobile phase consisting of 6% (v/v) (0.5% (v/v) trifluoroacetic acid) methanol-modified CO_2 at a pressure of 230 bar and a liquid flow rate of 2.0 mL/min. The purpose of the trifluoroacetic acid was to eliminate peak tailing of a possible degradation product, hydroxy acid lovastatin. The peak shape of lovastatin was not affected by the addition of the acidic additive to the mobile phase. [12] The column was maintained at 45 °C. The injection solvent consisted of methanol at a volume of 5 μL. Detection was by UV at 230 nm. A UV flow cell maintained at room temperature with 10 μL volume was used.

Traditional Liquid Extraction

Approximately 100 mg of the in-house tablet powder mixture or a MEVACOR® tablet was placed into a 50 mL volumetric flask. Then, 10 mL of an acetic acid- sodium acetate buffer (pH=4.0) was added to the flask, and the solution was sonicated until the tablet powder/tablet was fully disintegrated (15 minutes). Next, 35 mL of acetonitrile was added to the flask, and the solution was sonicated for 20 minutes. After cooling to room temperature (30 minutes), the solution was diluted to 50 mL with acetonitrile. Analyses were performed by SFC on the resulting solutions.

In-House Tablet Powder Mixture

The in-house tablet powder mixture was prepared by mixing all the ingredients except for lovastatin in a round bottom flask. Lovastatin was then dissolved in methanol (50 mL) and added to the flask with stirring. The tablet mixture was refrigerated overnight. The methanol was then removed by rotary evaporation. The concentration of

[12] *J.T.B. Strode, L.T. Taylor, A.L. Howard, D. Ip, M.A. Brooks*, "Analysis of Lovastatin by Packed Column Supercritical Fluid Chromatography", submitted for publication.

lovastatin in the in-house tablet powder mixture was 10 mg lovastatin/100 mg tablet powder. Four samples were taken to test uniformity of the tablet mixture (**Table 3.2**) using the traditional liquid extraction method, followed by SFC analysis.

MEVACOR® Tablet Crushing Method

Each commercially prepared MEVACOR® tablet (Merck Research Laboratories, West Point, PA) was placed on top of a piece of weighing paper which was loose in a mortar cup. A pestle was placed on top of the tablet, and pressure was applied until the tablet particles appeared evenly dispersed as a powder. The weighing paper was carefully removed and the complete crushed tablet was poured into the extraction vessel filled approximately 3/4 with a cotton ball. The weighing paper, mortar, and pestle were wiped clean with an additional small piece of cotton. This particular piece of cotton was then placed on top of the other cotton ball inside the extraction vessel. More cotton was added to fill approximately 90% of the vessel volume. The extraction vessel was then sealed.

3.3 RESULTS AND DISCUSSION

Phase A - Effect of Methanol-Modifier Concentration

Lovastatin, an antihypercholesterolemic drug (**Figure 3.1**), was chosen as the test analyte because it is relatively polar and exhibits marginal solubility (0.04 % (w/w) at 5000 psi, 40 °C) in 100% CO_2. [4] Larson and King found that the solubility was dramatically increased to 0.4% (w/w) with the incorporation of 5% (w/w) methanol-modified CO_2 from a pre-mixed tank. The increased solubility of lovastatin in the methanol-modified CO_2 versus pure CO_2 was attributed to the ability of the dilute methanol to hydrogen bond with the polar lovastatin. Consequently, a series of extractions (**Table 3.1**) were performed to determine the extractability of lovastatin from the in-house prepared tablet

Table 3.2. In-house Prepared Tablet Powder Mixture Uniformity With Liquid Extraction Method

Sample #	mg Lovastatin/100 mg tablet powder
1	9.78
2	9.33
3	9.50
4	9.60
Average	9.55
% RSD	2.0

SFC Conditions Used for Tablet Powder Uniformity. Column: Hypersil® silica (4.6 mm i.d. X 25 cm, 3 μm particle diameter), Mobile Phase: 6% (v/v) (0.5% (v/v) trifluoroacetic acid) methanol-modified CO_2; Pressure: 230 bar; Column Temperature: 45 °C; Liquid Flow Rate: 2.0 mL/min; Injection solvent: methanol; Injection Volume: 5 μL; Detection: UV at 230 nm. UV Flow Cell Volume, Temperature: 10 μL, room temperature.

Liquid-solid extractions - see Experimental for procedure

powder mixture with methanol-modified CO_2. Experiments were designed in such a way that an extraction profile could be constructed from the data (**Figure 3.2**) in order to examine the effect of methanol concentration and to investigate the extraction kinetics of lovastatin. A series of dynamic mini-extraction steps followed by trap rinsing and assay was employed. The tablet powder mixture was first allowed to equilibrate with the fluid for a certain period of time (static time) followed by continuous CO_2 flow for an additional period of time (dynamic time). The constructed extraction profiles consisted of several dynamic ministeps whereby the extraction was placed in static mode after a certain dynamic period, and the solid-phase trap was rinsed and assayed for percent recovery for that particular dynamic period. A tandem solid-phase/liquid trap was employed to ensure quantitative trapping recovery. Lovastatin recoveries were found to be low over the first forty minutes where only 58% was extractable with 1% (v/v) methanol-modified CO_2, and 77% was extractable with 5% (v/v) methanol-modified CO_2. When utilizing 1% and 5% (v/v) methanol-modified CO_2, the extraction profile suggested that most of the extractable lovastatin was removed during the first 20 minutes of dynamic extraction. During this period, the extraction appeared to be dependent upon the solubility of the analyte in the methanol-modified CO_2. After 20 minutes, the extraction process appeared to be limited by the diffusion of the analyte from the matrix into the SF. [13] Quantitative recoveries from the in-house prepared tablet powder mixture of greater than 97% were, however, achieved with 10% (v/v) methanol-modified CO_2 employing dynamic extraction mini-steps (**Figure 3.2).** Since trapping becomes more difficult with modifier concentrations greater than 2%, it was of interest to determine if the addition of a secondary modifier could reduce the primary modifier concentration.

[13] *K.D. Bartle, A.A. Clifford, S.B. Hawthorne, J.J. Langenfield, D.J. Miller, R. Robinson*, J. Supercrit. Fluids, **3** (1990) 143.

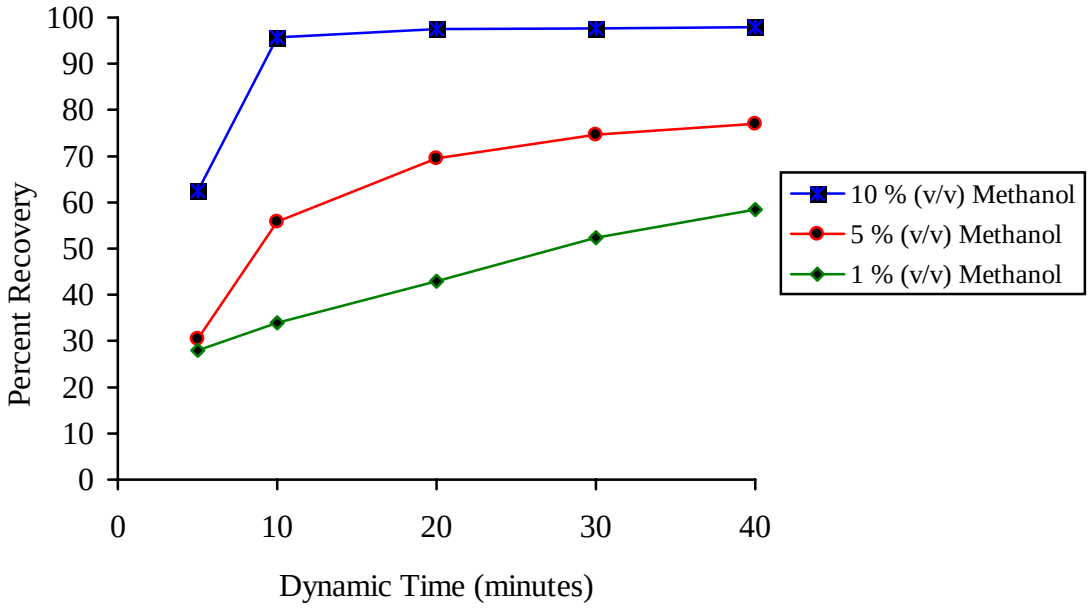


Figure 3.2. Effect of Methanol-Modifier Concentration on Lovastatin Recoveries (n=3) From In-House Prepared Tablet Powder Mixture

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Initial Static Time: 3.0 min.; Dynamic Time: 40.0 min. (total of 5 dynamic mini-steps); Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step.

Sample - 100 mg tablet powder mixture containing 10 mg lovastatin

Phase B - Effect of Additive Composition

After determining the effect of methanol on extraction efficiency, the role of the secondary modifier (i.e. additive) type was investigated. Methanol-modified CO_2 (1% (v/v)) was chosen as the extraction fluid in Phase B due to the limited extractability of lovastatin under these conditions so that the apparent effect of each additive on the extractability could be ascertained. Each additive was introduced directly to the methanol as 1% (v/v). The total additive concentration introduced corresponds to 0.0001%. It can be seen in **Figure 3.3** that isopropylamine was the only additive that significantly improved the extractability of lovastatin over time from the prepared tablet powder mixture. In fact, similar extraction recoveries utilizing all three additives (i.e. acid, neutral, base) were observed during the first 20 minutes of the extraction. During this time, the extraction was apparently governed simply by the solubility of the lovastatin in the 1% (v/v) methanol-modified CO_2. The extractability of lovastatin, however, increased from 58% with 1% (v/v) methanol-modified CO_2 to 71% with 1% (v/v) (1% (v/v) isopropylamine) methanol-modified CO_2 after 40 minutes. T-tests were performed in order to statistically compare the average extraction recoveries after 40 minutes of all three additives with methanol vs methanol-modified CO_2 alone. With a 95% confidence interval, it was shown that the extraction recoveries of lovastatin (e.g. after 40 minutes)
were statistically greater with the use of isopropylamine rather than with trifluoroacetic acid and tributylphosphate or no additive at all.

The increased extractability of lovastatin with the secondary modifier, isopropylamine, after 40 minutes can not simply be explained by enhanced solubility, but by a combination of solubility and analyte displacement from the matrix. Excluding the active drug substance, common tablet ingredients include filling agents such as cellulose and starch as well as lubricants and coloring agents. Cellulose, for example, contains free methoxy and hydroxy acidic sites which contribute to the “activity” of the matrix. Lovastatin, containing a lactone ring (cyclic ester), may be considered basic due to

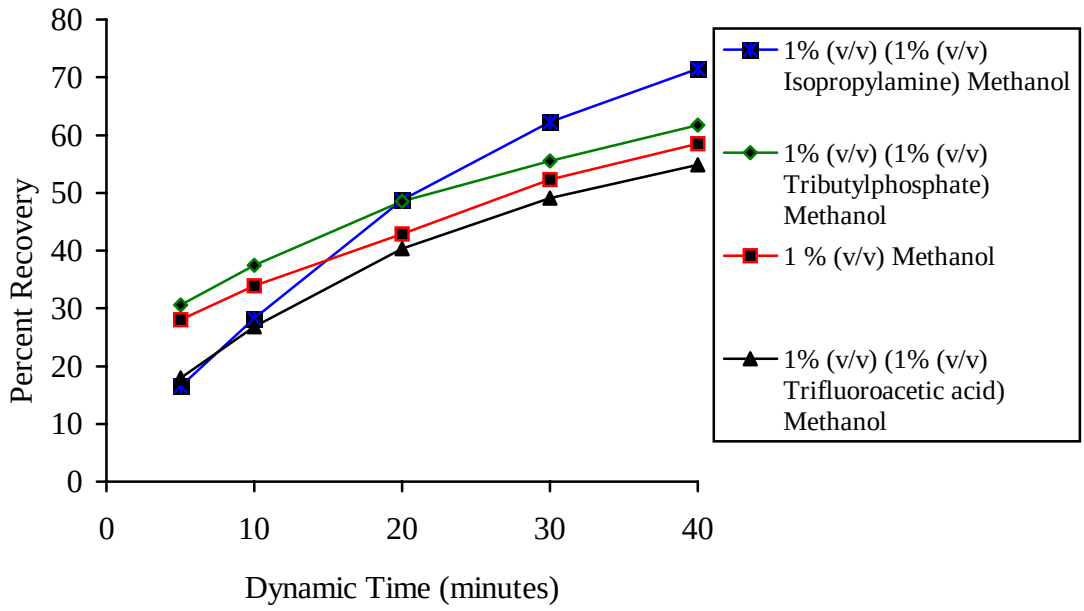
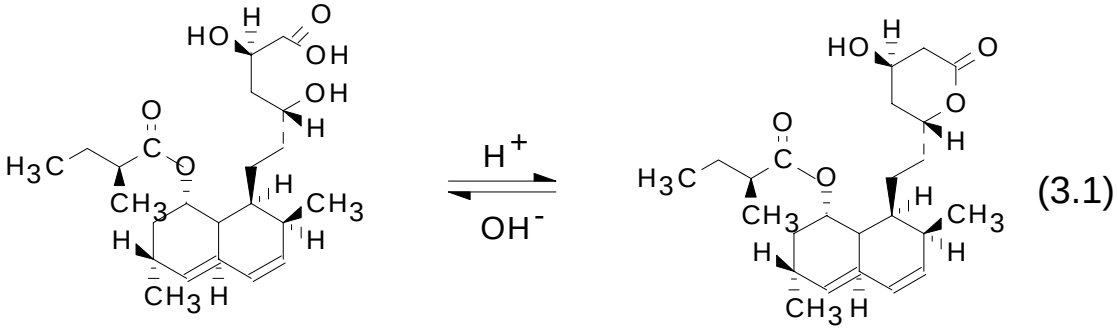


Figure 3.3. Effect of Additive Type on Lovastatin Recoveries (n=3) From In-House Prepared Tablet Powder Mixture

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Initial Static Time: 3.0 min.; Dynamic Time: 40.0 min. (total of 5 dynamic mini-steps); Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step

Sample - 100 mg tablet powder mixture containing 10 mg lovastatin

unshared pairs of electrons on the oxygen in the lactone ring, as well as its ability to accept protons. When treated with base, lactone rings are known to open up due to hydrolysis of the cyclic ester. Specifically, Larson et al. report the conversion of lovastatin in fermentation broth to its hydroxyacid form when in the presence of 3% methanol-modified CO_2 and t-butylamine (**Equation 3.1**). [4]



(3.1)

Hydroxyacid lovastatin Lovastatin

Knowing that lovastatin is basic and that the tablet matrix contains many acidic sites, the enhanced extractability of lovastatin from the tablet powder mixture with the basic additive can be explained by displacement. In this case, when the basic additive was introduced, the stronger base, isopropylamine, preferentially adsorbed to the matrix thus displacing the basic analyte, lovastatin, from any acidic sites on the tablet powder matrix. The conversion of lovastatin to its hydroxyacid degradate during the extraction with isopropylamine was indeed a concern. However, when the SFC analysis which has the capability to separate lovastatin and its hydroxyacid degradate was performed, no additional chromatographic peaks were detected. Therefore, it was assured that the extracted lovastatin was present in the lactonized form. This was expected due to the low amounts of isopropylamine used (0.0001% (v/v)).

Phase C - Effect of Additive Concentration

Since the lovastatin extraction recoveries from the tablet powder mixture were shown to be statistically greater with isopropylamine than the extraction recoveries achieved with the other additives and methanol-modified CO_2 alone, the effect of isopropylamine concentration at various methanol-modified CO_2 concentrations was investigated further in Phase C. Surprisingly, increased additive concentrations (0.5%, 1.0%, 2.0% (v/v) in 1% (v/v) methanol-modified CO_2) at a constant modifier concentration did not affect the extraction recoveries or the extraction rate. It was believed that all matrix acidic sites were occupied by isopropylamine at a concentration of 0.5% (v/v) in methanol; therefore increased additive concentrations would not further increase lovastatin extractability.

The usefulness of the isopropylamine additive at various methanol-modified CO_2 concentrations can be also observed in **Figure 3.4**. T-tests were performed to compare the average extraction recoveries after 40 minutes with and without isopropylamine in 5% (v/v) methanol-modified CO_2. With a 95% confidence interval, it was shown that the extraction recoveries of lovastatin (e.g. after 40 minutes) were statistically greater when isopropylamine was used. Once again lovastatin extraction recoveries at various modifier concentrations were significantly enhanced with the presence of isopropylamine. Overall extraction recoveries over 40 minutes increased from 77% with 5% (v/v) methanol-modified to 86% with 5% (v/v) (1% (v/v) isopropylamine) methanol-modified CO_2.

Phase D - Extraction of MEVACOR® Tablets

A MEVACOR® tablet containing 10 mg of lovastatin was crushed, placed in an extraction vessel filled with cotton, and extracted under similar conditions as described in Phases A-C. The total extraction time was extended to 87 minutes (17 minutes total static time, 70 minutes total dynamic time) for 5% (v/v) methanol-modifier with and without

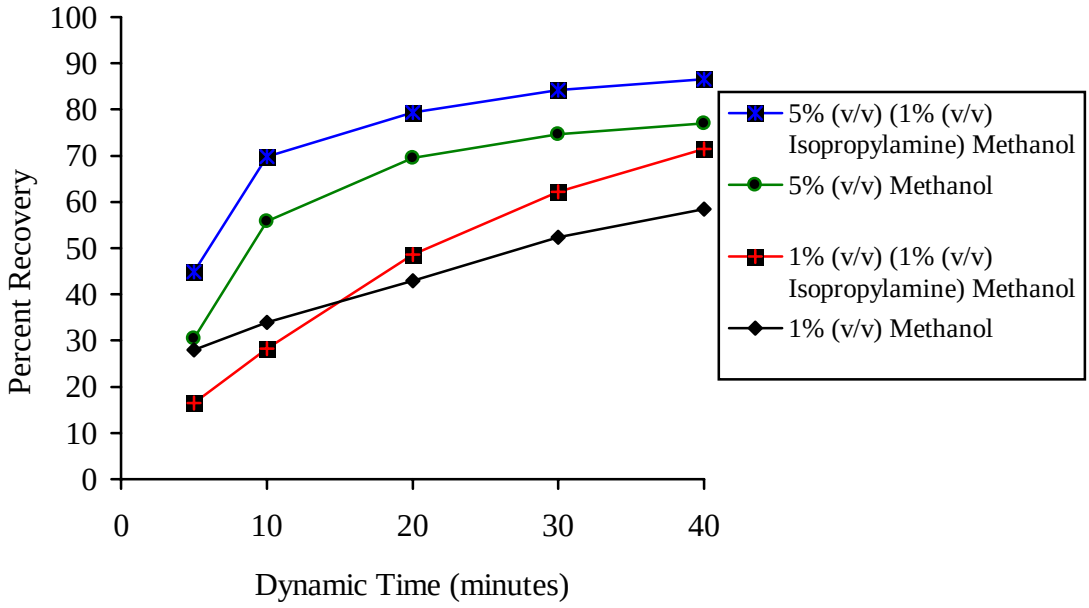


Figure 3.4. Effect of Methanol-Modifier Concentration on Lovastatin Recoveries (n=3) From In-House Prepared Tablet Powder Mixture

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Initial Static Time: 3.0 min.; Dynamic Time: 40.0 min. (total of 5 dynamic mini-steps); Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step.

Sample - 100 mg tablet powder mixture containing 10 mg lovastatin

isopropylamine (**Figure 3.5**). Since the additive concentration in methanol had no statistical effect on recovery, 1% (v/v) isopropylamine was chosen. An overall recovery of only 84% was achieved with 5% (v/v) (1.0% (v/v) isopropylamine) methanol-modified CO_2 within an extraction time of 40 minutes (dynamic); however, 106% was recovered within 70 minutes (dynamic). A MEVACOR® tablet was also extracted with 5% methanol-modified CO_2 (e.g. no isopropylamine) where only 74% was recovered within 70 minutes (dynamic). The advantages of the addition of isopropylamine as an additive when extracting from the MEVACOR® tablet were clearly shown.

Although quantitative lovastatin recoveries from MEVACOR® were achieved with 5% (v/v) methanol (1 % (v/v) isopropylamine), the time required for the extraction was 87 minutes (17 min. total static time, 70 min. total dynamic time). A dynamic extraction without trap rinsing between dynamic mini-steps as well as an extraction time of approximately 30 minutes was desired for the final optimized SFE method. Similar to the previous studies, extraction profiles consisting of alternating static/dynamic steps with trap rinsing in between each dynamic step were performed in order to compare overall extraction recoveries achieved and time needed versus the various modifier and additive percentages. A modifier percentage of 10% (v/v) methanol with and without isopropylamine was then investigated (**Figure 3.6**). Overall extraction recoveries (n=1) of 95 and 88% were achieved with 10% methanol with and without isopropylamine respectively, but the time needed was 50 minutes (6 dynamic mini-steps). Further attempts were made to increase the extraction recovery to 100% and to reduce the time needed to approximately 30 minutes. Therefore, 15% (v/v) methanol with and without isopropylamine was investigated (**Figure 3.7**). Once again an enhancement was observed when isopropylamine was employed where 102% and 91% with and without isopropylamine respectively was recovered (n=1), and in this case, the extraction time needed with the isopropylamine was 35 minutes (static and dynamic). Percent lovastatin recovery comparisons as a function of methanol-modifier percentage, dynamic time needed, and addition of isopropylamine are found in **Table 3.3**.

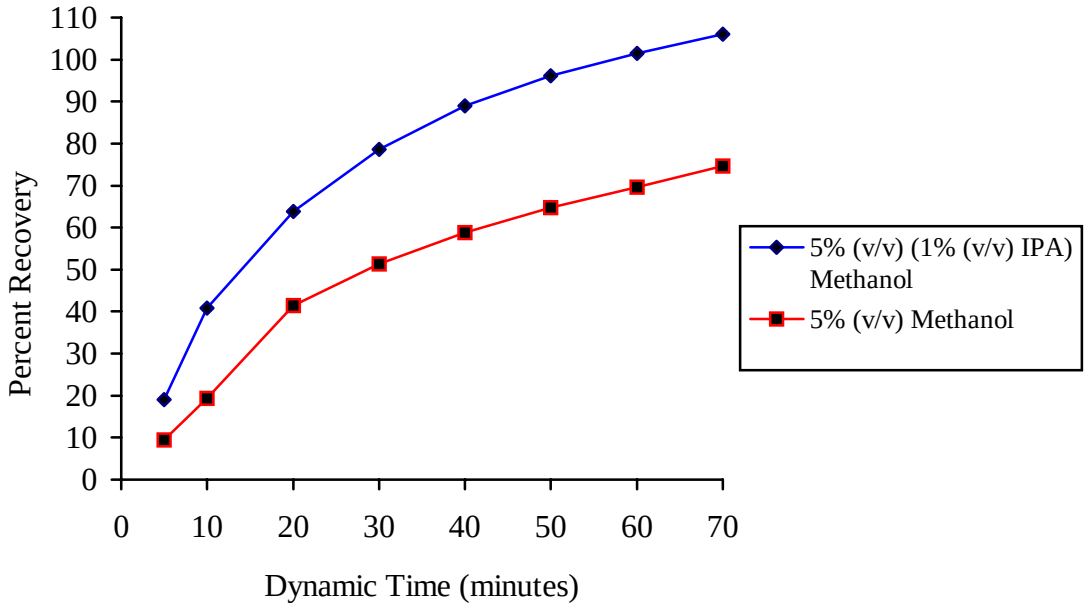


Figure 3.5. Subcritical Fluid Extraction (n=1) of Lovastatin From MEVACOR® Tablets at Various Additive/Modifier Concentrations

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Static Time: 3.0 min.; Dynamic Time: 70.0 min. (total of 8 dynamic mini-steps); Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step.

Sample - 1 crushed MEVACOR® tablet containing 10 mg lovastatin

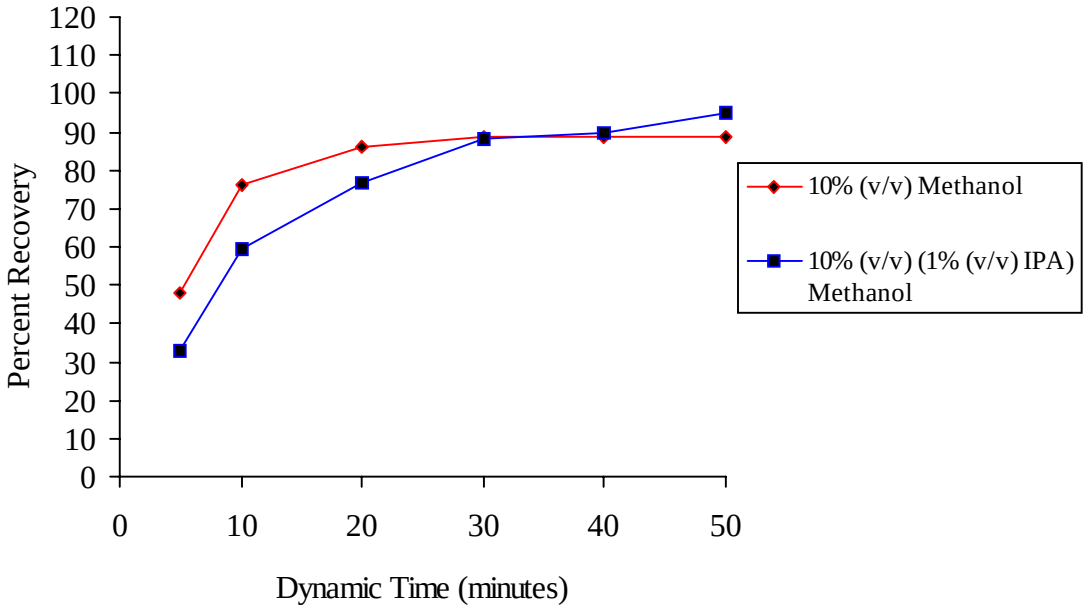


Figure 3.6. Subcritical Fluid Extraction (n=1) of Lovastatin From MEVACOR® Tablets at Various Modifier Concentrations With and Without isopropylamine

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Static Time: 3.0 min.; Dynamic Time: 50.0 min. (total of 6 dynamic mini-steps); Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step.

Sample - 1 crushed MEVACOR® tablet containing 10 mg lovastatin

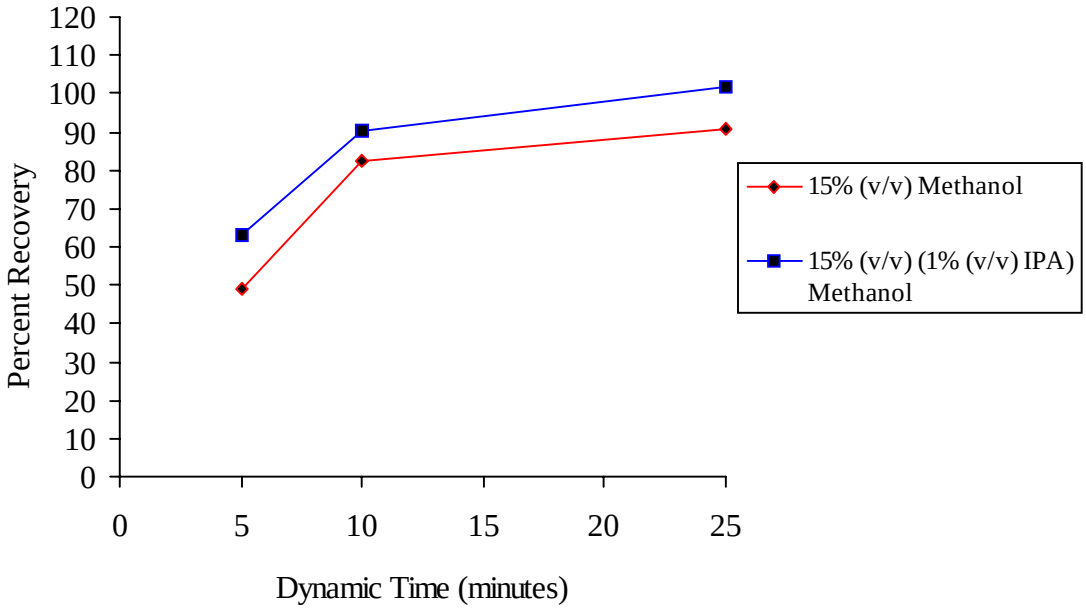


Figure 3.7. Subcritical Fluid Extraction (n=1) of Lovastatin From MEVACOR® Tablets at Various Modifier Concentrations With and Without isopropylamine

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Static Time: 3.0 min.; Dynamic Time: 25.0 min. (total of 3 dynamic mini-steps); Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step.

Sample - 1 crushed MEVACOR® tablet containing 10 mg lovastatin

Table 3.3. SFE of MEVACOR® Tablets as a Function of Methanol-Modifier Percentage and Addition of Isopropylamine

% Methanol-Modified CO_2	Dynamic Time (min.)	% Recovery no Isopropylamine (n=1)	% Recovery (1% (v/v) Isopropylamine in Methanol) (n=1)
5%	70	84	106
10%	50	88	95
15%	25	91	102

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 5 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 2.0 mL, Solid-Phase Rinsing Flow Rate: 1.0 mL/min.; Static Time: 3.0 min.; Dynamic Time: see table; Static Time During Trap Rinsing: 2.0 min. *Solid-phase trap rinsed directly into tandem liquid trap following each dynamic step.

In the belief that an optimized method had been developed, 5 MEVACOR® tablets were then extracted with 15% (v/v) methanol with 1% (v/v) isopropylamine. The extraction method consisted of 3 dynamic mini-steps with a 2 minute static time added between each dynamic step to mimic trap rinsing as was used when constructing the previous extraction profiles. In this case, the solid-phase trap was not rinsed until the 35 min. extraction was completed. Average percent recoveries (n=5), standard deviations, and % RSDs are found in **Table 3.4**. It can be seen that 10 mg of lovastatin per tablet was fully recovered (99.5%) from the MEVACOR® tablets with a % RSD of 1.2% with 15 % (v/v) (1% (v/v) isopropylamine) methanol-modified CO_2 within 35 minutes. As compared to the traditional liquid extraction procedure (**Table 3.2**), the SFE method has been shown to be very advantageous (**Table 3.5**). The use of acetonitrile and buffer has been eliminated, and solvent consumption has been reduced from 95 mL to 17.5 mL of methanol consisting of: modifier (7.5 mL), tandem liquid trap (5 mL), and solid-phase rinsing (5 mL). Also many laborious and time consuming steps performed in the liquid extraction such as the addition of buffer and acetonitrile, mixing, sonicating, and cooling steps have been eliminated. As compared to the traditional liquid extraction procedure, the extraction time was reduced from over an hour to merely 35 minutes by using SFE. All that is required for the SFE method is crushing the tablet, placing it in the extraction vessel, and performing the one-step extraction.

Table 3.4. SFE Reproducibility for Lovastatin (10 mg) from MEVACOR® Tablets with 15 % (1.0% (v/v) isopropylamine) Methanol-Modified CO_2

Tablet #	Percent Recovery
1	98.4
2	101.0
3	98.7
4	98.7
5	100.5
Avg. Percent Recovery	99.5
RSD	1.2

SFE Conditions. CO_2 Pressure: 400 atm; Oven Temperature: 40 °C; Liquid Flow Rate: 2.0 mL/min.; Restrictor Temperature: 50 °C; Solid-Phase Trap: 50/50 (w/w) Porapak Q/Glass Beads; Liquid Tandem Trap: Methanol; Liquid Tandem Trap Volume: 7 mL; Collection Temperature (Solid-Phase): 40 °C, Desorption Temperature (Solid-Phase); 40 °C; Solid-Phase Rinse Solvent/Volume: Methanol, 5.0 mL, Solid-Phase Rinsing Flow Rate: 2.0 mL/min.; Static Time: 3.0 min.; Dynamic Time: 25.0 min. (total of 3 dynamic mini-steps); Static Time Between Dynamic Steps: 2.0 min.

Table 3.5. Traditional Liquid Extraction and SFE Solvent Use, Steps, and Time Comparisons

	Traditional liquid extraction	**SFE**
Volume of Solvent Used	95 mL acetonitrile/buffer	17.5 mL methanol total • 7.5 mL modifier • 5 mL tandem-liquid trap • 5 mL solid-phase rinse
Mixing	XXX	none
Sonication	XXX	none
Cooling	XXX	none
Tablet Crushing	none	XXX
Extraction Time Including Sample Preparation	>60 min.	35 min.

3.4 SUMMARY

The effect of primary and secondary modifiers (i.e. additives) on the subcritical fluid extraction of lovastatin from in-house prepared tablet powder mixtures and MEVACOR® tablets was investigated. Methanol-modifier percentage, additive type (acidic, basic, neutral) in methanol, and the effect of additive concentration on the extraction efficiency were examined. Extractability was shown to depend on modifier concentration and additive type. Isopropylamine was believed to be the most successful additive because of its ability to displace adsorbed lovastatin from the acidic tablet matrix sites, an effect not possible with methanol-modified CO_2 alone. An optimized extraction method was developed, and lovastatin recoveries of 99.5% with a RSD of 1.2% from MEVACOR® tablets with 15% (v/v) (1.0% (v/v) isopropylamine) methanol-modified CO_2 was achieved.