Pergamon

0731-7085(94)00048-4

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 8, pp. 1003–1014, 1994
Elsevier Science Ltd
Printed in Great Britain
0731-7085/94 $7.00 + 0.00

Analysis of felodipine by packed column supercritical fluid chromatography with electron capture and ultraviolet absorbance detection

J.T.B. STRODE III, L.T. TAYLOR, A.L. HOWARD,*† D. IP† and M.A. BROOKS†

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
†Merck Research Laboratories, West Point, PA 19486, USA

Abstract: A reproducible and selective supercritical fluid chromatography (SFC) method was developed for the analysis of felodipine, a drug indicated for the treatment of hypertension. Methanol-modified carbon dioxide was employed as the SFC mobile phase with both electron capture detection (ECD) and multi-wavelength detection (MWD) being used simultaneously for analyte determination. Chromatographic limit of detection (LOD) and limit of quantitation (LOQ), linear dynamic range (LDR) and injection precision were obtained in order to assess chromatographic and detector performance for both the SFC/MWD and SFC/ECD/MWD systems. The method was shown to be stability indicating since felodipine could be separated from its potential oxidative degradation product, H152/37, in under 6 min (felodipine $k' = 2.44$). Sample throughput was increased by 60% with the SFC assay vs LC. The optimized SFC method was shown to be equivalent to an existing LC/UV procedure for the analysis of a sustained-release tablet while realizing a 92% saving in disposable solvent waste. In order to achieve further solvent savings overall, supercritical fluid extraction (SFE) with 8% methanol-modified carbon dioxide as the extraction fluid was used to extract felodipine from a sustained-release tablet (as opposed to traditional solvent extraction). Comparable drug recoveries were obtained with SFE sample preparation technique when either SFC or LC extract analysis was utilized.

Keywords: *Felodipine; felodipine oxidative degradation; packed column supercritical fluid chromatography; electron capture detection; methanol-modified carbon dioxide mobile phase.*

Introduction

Traditionally, the pharmaceutical industry has employed reversed-phase liquid chromatography (LC) with UV absorbance detection as the analytical method of choice. In most cases, due to the polar nature, high molecular weight and/or thermal lability of the analyte, this technique is required. However, in the case of felodipine (Fig. 1), a calcium antagonist of the dihydropyridine class for the treatment of hypertension, the use of LC is warranted only by the drug's thermal lability above 50°C. In spite of this characteristic, felodipine has been previously determined by gas chromatography (GC) with either electron capture [1–9], thermionic [10], mass spectrometric [8, 11–13], or flame ionization [14] detection. The compatibility of GC with these sensitive selective detectors makes its use attractive for the identification and quantitation of low level biological felodipine metabolites even though the moderate polarity and thermal instability of felodipine caused peak tailing [7] and oxidative degradation [7]. Some authors minimized degradation by silylating the injection liner [10] or by employing cold on-column injection techniques [7]. For the more polar carboxylic acid metabolites of felodipine, reversed-phase LC analysis was necessary [15]. Overall, more matrix interference peaks were observed in the analysis of plasma samples with the LC/UV [15] separation vs the analagous GC separation [1–14] thus further demonstrating the advantage of selective detection available for GC. However, for the analysis of formulated felodipine products, GC is unacceptable since it has the potential to degrade the drug.

For the analysis of formulated felodipine product (tablets), reversed-phase LC/UV is currently used since any co-extracted high molecular weight and/or highly polar excipients are compatible with the chromatographic system. UV detection is ideal for this analysis since co-extracted excipients from the

*Author to whom correspondence should be addressed.

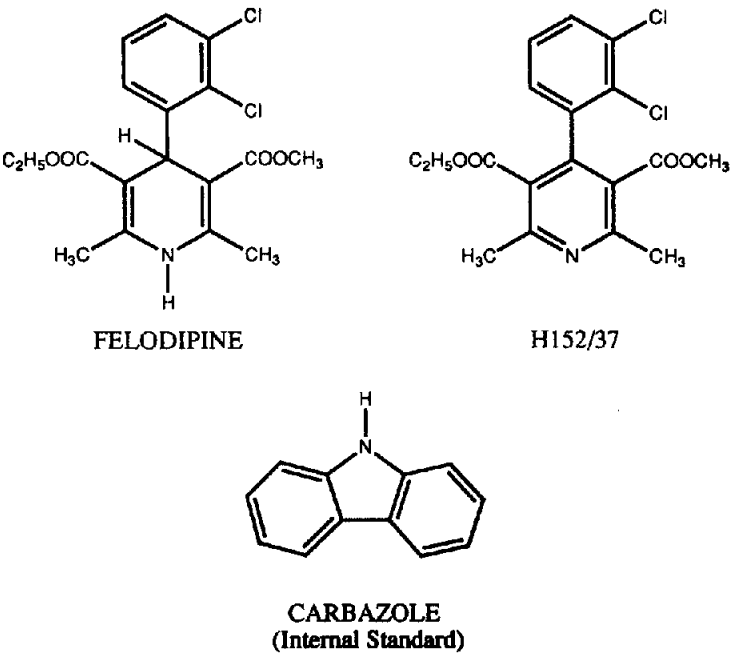

Figure 1
Chemical structure for felodipine and its pyridine derivative, H152/37.

tablet matrix do not contain chromophores. However, LC itself is not attractive from the standpoint of solvent disposal. In many cases, the main source of disposable solvent waste generated in the pharmaceutical laboratory results from HPLC analysis. In general, the disposal of reversed-phase LC (organic/water mixtures) mobile phases is much more expensive ($175/55 gal.) than disposing of 100% organic waste ($48/55 gal.) due to low energy output upon combustion (Solid Waste Management, Merck Research Laboratories, West Point, PA, USA).

Therefore, for a compound such as felodipine, the ideal chromatographic technique would be: (1) thermally mild; (2) interfaceable with LC-type detection (UV or MWD); (3) compatible with selective GC detection; and (4) generate less disposable solvent waste. Supercritical fluid chromatography (SFC) with carbon dioxide-based mobile phase meets these requirements. SFC has been reported in the literature recently for the analysis of many pharmaceutical agents including retinol palmitate/tocopherol [16], free bile acids [17], benzodiazepines [18, 19], non-steroidal anti-inflammatory agents [20], sulphonamides [21, 22], opium alkaloids [23] found in the following matrices: animal tissue [24]; natural products [23]; and formulated drug products [16, 17, 19, 20]. In these investigations, the previously mentioned advantages of SFC analysis were demonstrated. Enhanced chromatographic efficiency of SFC due to the low viscosity and high diffusivity of the mobile phase was also illustrated (vs HPLC analysis). The analysis of felodipine or other dihydropyridine calcium antagonists by SFC has not been previously reported to date in the literature.

The recent improvement in two major components of commercial SFC instrumentation has also made routine SFC analysis in the pharmaceutical laboratory more feasible. First, the use of variable restriction has significantly improved system stability. In older SFC equipment, a narrow bore and/or tapered fused silica tube was used to maintain system pressure. With such a fixed restrictor, any changes in system pressure resulted in a change in mobile phase flow rate (linear velocity) since the restrictor i.d. was fixed. With variable restriction, the restrictor responds to changes in system pressure so that mobile phase flow rate and, therefore, linear velocity remain constant. Second, the availability of on-line organic modifier addition to the SF mobile phase allows reproducible analysis of moderately polar analytes such as pharmaceutical agents. In addition, method development is more flexible since SF composition (modifier percentage) and/or modifier identity can be easily changed. Both system improvements

have made SFC inherently more reproducible, accurate and precise for the determination of polar substances such as felodipine.

The results presented herein demonstrate several practical aspects and advantages of SFC vs LC analysis [25] for the determination of a moderately polar drug, felodipine. In order to illustrate these points, a rapid and selective SFC method was developed for the separation of felodipine from a potential tablet degradation product and known metabolite [8, 26], H152/37 (Fig. 1). Simultaneous detection employing both a gas phase GC detector (ECD) and a typical LC detector (MWD) is demonstrated. Under optimized chromatographic conditions, detector performance was assessed for both the SFC/MWD and SFC/ECD/MWD systems. In addition, accuracy, precision, analysis time and solvent usage comparisons were made for SFC/MWD vs LC/UV analysis of a sustained-release felodipine tablet.

Experimental

Chemicals

All solvents (methanol, acetonitrile, and water) (Fisher Scientific, Pittsburgh, PA, USA) were of LC purity. Pure felodipine drug and H152/37, felodipine oxidation product, were provided by Merck Research Laboratories, West Point, PA, USA (Fig. 1). Sustained-release felodipine tablets were also provided by Merck Research Laboratories. Carbazole (Aldrich) was used as the internal standard for quantitation of felodipine in tablets.

SFC instrumentation

A prototype of the Hewlett–Packard Model G1205 SFC system (Hewlett–Packard, Little Falls, DE, USA) (Fig. 2) was used for all SFC separations. With this SFC system, a Peltier-cooled reciprocating pump was operated in the flow control model (±0.001 ml min^{-1} liquid) to deliver SFC grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, USA) to the system at a flow rate of 2 ml min^{-1}. Mobile phase flow rate was measured as a liquid at the pump. When organic modifier was utilized, it was added on-line via an auxiliary reciprocating pump. The inlet pressure immediately downstream of the CO_2/modifier mixing chamber was monitored by an electronic pressure gauge. A Rheodyne Model 7410 air-actuated valve equipped with an autosampler and a 5-µl loop was used to introduce sample to a 25 cm × 4.6 mm i.d. Hypersil silica (Si) column (d_p = 5 µm) (Keystone Scientific, Inc., Bellefonte, PA, USA) which was located in a gas chromatographic-type oven. In order to perform simultaneous ECD and MWD

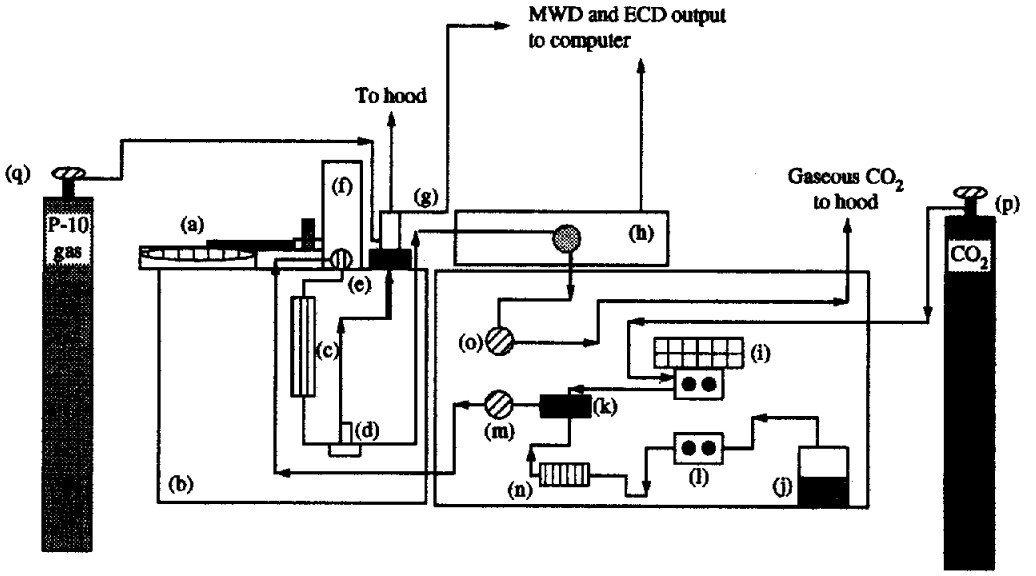

Figure 2
Hewlett–Packard SFC/ECD/UV system. Components are as follows: (A) autosampler; (B) oven, (C) 4.6 mm i.d. column; (D) splitting tee; (E) integral restrictor; (F) injection valve; (G) ECD; (H) MWD; (I) CO_2 reciprocating pump; (J) organic modifier reservoir; (K) check valve; (L) modifier reciprocating pump; (M) pulse dampener; (N) mass flow sensor; (O) variable restrictor; (P) CO_2 reservoir; and (Q) P-10 ECD auxiliary gas.

detection, a column eluent split was employed. The larger portion of the column eluent split was directed to a standard HP Model 1050 multi-wavelength detector (MWD) with a high pressure flow cell (13-μl volume) via a 1 m piece of tubing (0.17 mm i.d.). The remaining column eluent was transported to an HP Model 19233 ^{63}Ni electron capture detector via an integral restrictor (Supelco, Inc., Bellefonte, PA, USA). The detector temperature was held at 100°C and 5% methane in argon (P-5 gas) (Airco, Radford, VA, USA) at a flow rate of 400 ml min^{-1} was employed as the auxiliary gas in the ECD. Both detector signals were monitored by an HP ChemStation. A maximum of three MWD wavelengths and two GC detector signals could be monitored simultaneously by the ChemStation. Overall system pressure was maintained by a low dead volume, computer-controlled electronic back pressure regulator located at the exit of the MWD. This device allowed for pressure and flow rate to be controlled independently. The pressure given for all chromatograms was the respective outlet pressure.

LC equipment

LC analysis [25] was performed with an HP Model 1050 isocratic HPLC pump connected to a Valco valve equipped with an external 20 μl injection loop. All injections were made manually. A 25 cm × 4.6 mm i.d. Hypersil C_{18} column (d_p = 5 μm) (Keystone Scientific, Bellefonte, PA, USA) and an acetonitrile–methanol–50 mM potassium phosphate buffer (adjusted to pH 3 with phosphoric acid) (40:20:40, v/v/v) mobile phase at a flow rate of 1.5 ml min^{-1} were used to achieve analyte separation. Injection solvent used for chromatographic performance assessment was mobile phase. Felodipine and H152/37 were detected at 254 nm with a Kratos Model 757 variable wavelength dtector (Ramsey, NI, USA). Detector output was collected with an HP Model 3394A integrator. For tablet analysis, all quantitation was based on peak area ratios of felodipine standard/internal standard (carbazole). Any future references to the LC analysis of felodipine are based upon the method described here [25].

SFE equipment

An HP Model 7680T SFE unit was used to remove the drug from the crushed 5 mg potency felodipine tablet. Its operation was as follows. SFC grade pre-mixed 8% (w/w) methanol-modified CO_2 (Scott Specialty Gases, Plumsteadville, PA, USA) entered the extractor from any of three tank reservoirs. From the tank, the fluid proceeded to a cryogenically cooled (5°C) dual head reciprocating pump which was capable of pressures up to 5100 psi, flow rates up to 4 ml min^{-1} and densities up to 0.9 g ml^{-1}. When the extraction program began, the fluid entered the extraction vessel located in a thermally controlled extraction chamber where it became supercritical. From the extraction chamber, the fluid passed through a variable restrictor which maintained the desired pressure and flow rate. After decompression (post-restrictor), the gaseous CO_2 was vented to waste while the extractables were deposited in a cryogenically-cooled trap containing 100-μm stainless steel beads. The restrictor and trap were independently thermally controlled. After the extraction, the analyte was recovered from the trap by rinsing it with methanol. The analyte, once dissolved in the rinse solvent, was transferred to a 2-ml glass vial containing the internal standard, carbazole. The collection vials were housed on a fraction collector. Due to the low capacity of the trap (between 2 and 4 mg of trapped material), all extractions were performed in a series of steps where the trap is rinsed after every step, thereby minimizing sample loss. A combination static (10 min) and dynamic (10 min) extraction for a total of 80 min (4 steps) at 45°C and 315 bar was used for tablet extraction. Static extraction was performed by pressurizing the extraction vessel with a fixed amount of CO_2 for a given time period while dynamic SFE employed a continuous stream of fresh SF flowing through the sample. Two 1.4-ml aliquots of methanol were used to rinse the trap after each extraction step. Each aliquot of rinse solvent was collected and assayed separately. Carbazole, the internal standard, was added to each collection vial prior to trap rinsing. All quantitations were performed on the basis of internal standard ratios.

Results and Discussion

The goal of this work was to develop a rapid, efficient SFC separation of felodipine and H152/37 with similar precision and accuracy as the currently used LC assay while reducing

disposable solvent waste. For most LC analyses, maximal accuracy and precision are achieved under isocratic, isothermal chromatographic conditions. Gradient elution is employed only if a suitable isocratic method cannot be developed. Analogously, for SFC, isothermal, isobaric and isocratic conditions were likewise chosen as goals for the felodipine separation. Since felodipine and its possible degradation product are moderately polar, the use of high density SF is warranted in order to maximize the mobile phase solvating power. In order to achieve this density, high pressure (300 bar) and low temperature (45°C) were initially thought to be the most applicable. In addition, the lower operating temperature was selected to prolong packed column lifetime and to minimize degradation of felodipine.

SFC optimization

Since analyte elution did not occur with pure CO_2 (300 bar, 45°C), the use of modifier was required for the analysis. The addition of small amounts of organic solvent to the CO_2 generally has the effect of (1) increasing mobile phase polarity and (2) covering active silanol sites on the column. With the addition of 1% methanol (v/v) CO_2, the drug eluted but with a retention time of 15 min and a poor peak shape, therefore indicating that higher modifier concentrations were necessary.

In order to further understand the effects of modifier on the SFC analysis of felodipine, 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). LC peak parameters were also calculated for the LC felodipine analysis and are given for comparison in Table 1. All values are based on five replicate felodipine injections. It is important to note here that the linear velocity was constant for each SFC experiment due to the electronically controlled back pressure regulator. From Table 1, it appears that the plate height was not significantly influenced by varying the modifier concentration from 4 to 8%. Conversely, the SFC capacity factor was significantly decreased as the modifier concentration was increased from 4 to 8%. This result was expected since the mobile phase polarity increased, thereby decreasing felodipine retention on the column. However, at similar k' values and column lengths (4% methanol-modified CO_2 SFC vs LC), plate height (h) was found to decrease by 45% while the number of plates/column (N) was found to increase by 37% for the analysis of felodipine. Peak width at half weight ($w_{1/2}$) also decreased by 43% with the SFC analysis. The decrease in plate height and peak width at half height and the increase in N demonstrates the gain in chromatographic efficiency afforded by the high mass transfer properties of the SF mobile phase as compared to the liquid mobile phases employed with LC. Peak shape (t_f) was excellent (1.0) with all SFC modifier concentrations of 4–8% v/v. It should be noted here that the lack of temperature control for the LC analysis can adversely affect the retention time reproducibility. The use of 6% (v/v) methanol-modified CO_2 was chosen for all further separations since it represented the best compromise between analysis time and column efficiency.

Table 1
Effect of modifier concentration on SFC analysis of felodipine

	Modifier concentration (volume %)			
	4%	6%	8%	LC*
Retention time (min)	7.03 (2.2%)	4.34 (0.3%)	3.29 (0.2%)	9.23 (0.5%)
t_0 (min)	1.35	1.26	1.28	1.52
k'	4.21	2.44	1.57	5.07
$w_{1/2}$ (min)	0.14	0.089	0.071	0.25
N	13535	13115	11722	8519
h (mm per plate)	0.016	0.017	0.019	0.029
t_f	1.0	1.0	1.0	1.2
Peak area RSD	1.3%	1.1%	1.8%	1.2%

SFC analysis conditions were as follows: 25 cm × 4.6 mm i.d. Hypersil Si column; temperature, 45°C; pressure, 300 bar; flow rate, 2 ml min^{-1}; injection volume, 5 µl. Felodipine concentration was 1 mg ml^{-1}.

† All peak parameters were calculated based upon five replicate injections of a felodipine standard. Where applicable, RSD values are given in parentheses.

*Conditions as described in the Experimental section.

Table 2
Effect of pressure on SFC analysis of felodipine

	SFC analysis pressure (bar)			
	80*	180	230	280
Retention time (min)	9.41	4.84	4.25	3.95
t_0 (min)	1.28	1.30	1.29	1.33
k'	6.35	2.72	2.29	1.97
$w_{1/2}$ (min)	0.43	0.15	0.12	0.11
N	2670	5767	6949	7001
t_f	1.03	0.92	0.87	0.95
h (mm per plate)	0.082	0.038	0.032	0.031

SFC conditions were as follows: Mobile phase, 6% (v/v) methanol–modified CO_2; temperature, 45°C; 25 cm × 4.6 mm i.d. Hypersil Si column; flow rate, 2 ml min^{-1}; injection volume, 5 μl. Felodipine concentration was 1 mg ml^{-1}.
*Peak splitting occurred.

The effect of pressure on the chromatographic peak parameters of felodipine was also examined (Table 2). It is important to note again that the linear velocity remained constant for each experiment as is evidenced by the constant t_o value. Generally, both plate height (h) and capacity factor (k') decreased with increasing pressure. Since density as well as mobile phase solvating power are proportional to pressure, lower felodipine retention time was expected with an increase in pressure. A minimum in h occurred between 250 and 280 bar. A pressure of 280 bar was chosen as the optimal operating pressure.

SFC/MDW detector performance

The implementation of UV (or MWD) detection in packed column SFC (4.6 mm i.d.) is identical to that in analytical scale LC. Chromophoric species are detected in the SF mobile phase prior to SF decompression (pre-restrictor). For the traditional HPLC method, felodipine was monitored at a different wavelength (362 nm) than that of H152/37 (254 nm) due to sensitivity differences. Using the conditions given in Fig. 3, MWD detector performance was assessed for the analysis of pure felodipine at both 254 and 362 nm in order to determine if dual wavelength analysis was necessary. The linear dynamic range for the SFC/MWD system was determined to be two orders of magnitude (18–5000 ppm) at both 254 (r = 0.9998) and 362 nm (r = 0.99997). Injection reproducibility based upon peak areas for SFC/MWD was found to be 1–3% RSD for replicate injections (n = 5) of individual samples at varying felodipine concentrations. The day-to-day injection precision was estimated to be 2.2% measured over 3 days.

Chromatographic limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the propagation of error method [27]. All pertinent calibration curve data are listed in Table 3. The terms in Table 3 (and 4) are defined as follows: m, calibration curve slope; i, intercept; r, correlation coefficient; s_m, standard deviation of slope; s_i, standard deviation of intercept; $s_{x/y}$, point error. Curves were constructed for SFC/MWD analysis alone as well as for the SFC/MWD/ECD system. At 254 nm for the SFC/MWD system, the LOD was found to be 12 ppm and the LOQ was determined to be 42 ppm. For the LOD and LOQ, respectively, this represents the introduction of 62 ng felodipine in the former and 208 ng felodipine in the latter onto the column via a 5-μl injection volume. At 362 nm, the LOD was calculated to be 5 ppm and the LOQ 18 ppm. For these LOD and LOQ values, 25 ng and 91 ng felodipine, respectively, were introduced to the column. For LC analysis with the previously described LC system (assuming the same UV detector geometry and felodipine molar absorptivity), the concentration LOD would be 3 ppm at 254 nm and 1 ppm at 362 nm as a result of the larger injection volume (20 μl).

From the comparison of the LOD and LOQ values for LC vs SFC, it appears that the small injection volume (1–5 μl) used in SFC is a disadvantage when compared to that typically used for LC (i.e. 5–250 μl). The difference in polarity between the injection solvent and the carbon dioxide-based mobile phases is the reason that smaller injection volumes are

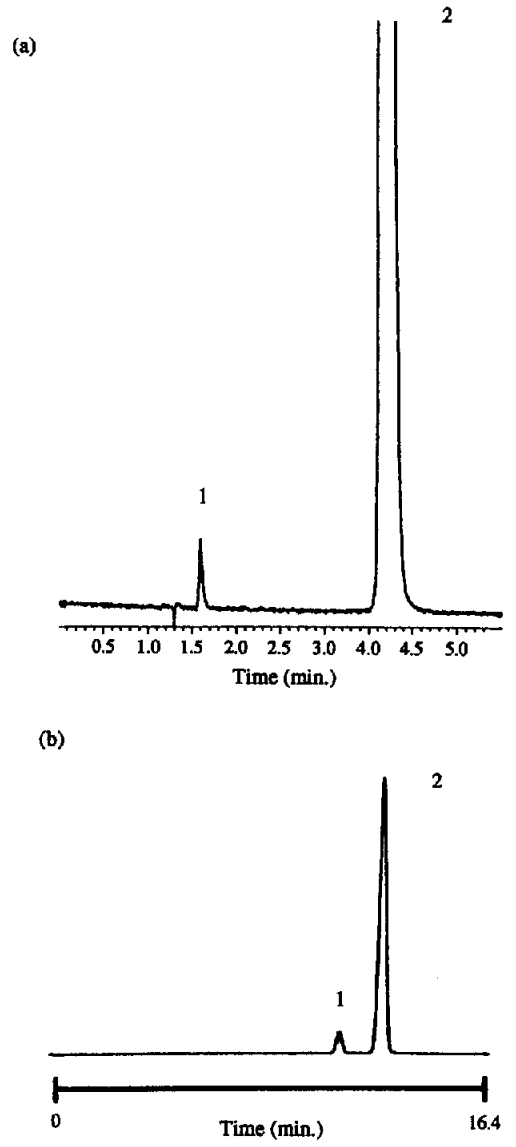

Figure 3
(A) SFC/UV and (B) LC/UV felodipine separation. SFC conditions: mobile phase, 6% (v/v) methanol-modified CO_2; pressure, 280 bar; temperature, 45°C; column, 25 cm × 4.6 mm i.d. Hypersil Si; flow rate (liquid), 2 ml min^{-1}; injection volume, 5 µl; injection solvent, methanol. LC/UV conditions: mobile phase, acetonitrile–methanol–0.05 M potassium phosphate buffer (adjusted to pH 3 with phosphoric acid) (40:20:40, v/v/v); injection volume, 20 µl; injection solvent, mobile phase; solution concentration, 0.1 mg ml^{-1}; temperature, ambient; column, 25 cm × 4.6 mm i.d. Hypersil C_{18}; flow rate, 1.5 ml min^{-1}; UV detection, 254 nm. Peak identity is as follows: (1) H152/37 and (2) felodipine.

typically employed in SFC. This conclusion may not be valid when the injection solutions required for each technique are examined, however. First, it is important to note that the best solution medium for felodipine would be an organic one (i.e. methanol), due to the drug's hydrophobic nature. For reversed-phase LC analysis, however, the injection of 100% organic solvent solutions is not recommended when aqueous/organic mobile phases are employed since peak splitting can occur. Therefore, organic sample solutions are usually diluted with water in order to match the solvent strength of the reversed phase LC mobile phase. On the other hand, SFC requires the injection of organic solvent solutions only. Therefore, no dilution would be necessary and higher drug solution concentrations are possible. Conversely, the need for organic solvent solutions can be a limitation with liquid tablet extraction since tablet disintegration can be problematic in a non-aqueous medium.

A typical packed column SFC/MWD separation of felodipine's oxidative degradate, H152/37 (Peak 1) and felodipine (Peak 2) is shown in Fig. 3(a). The degradation product (Peak 1) was generated *in situ* by heating a solution of felodipine at 80°C overnight. Its identity was confirmed to be H152/37 by injecting a solution of H152/37 prepared from reference material. A resolution (R_s) factor of 2.6 was obtained for the SFC separation vs 2.5 for the optimized HPLC analysis (Fig. 3 b). In general, the resolution of these two components with SFC was less sensitive to separation conditions than that seen in reversed-phase LC separation. In reversed-phase LC, the separation of these components was found to be highly dependent on the mobile phase composition and the age of the column. No such dependence was found in the case of SFC.

It is interesting to note that the elution order of the degradate and felodipine from the Hypersil Si column under SFC conditions (Fig. 3a) is identical to that seen when a C_{18} column was used under reversed-phase LC conditions (Fig. 3b). According to chromatographic theory, normal phase stationary phases should produce separations with opposite elution order vs their reversed-phase counterpart. It appears almost as if the retention of these two compounds is not influenced by stationary phase polarity when, in actuality, the mechanisms of retention may be influenced by different aspects of each molecule. For example, the more hydrophilic compound (H152/37) elutes first in reversed-phase LC since it partitions more favourably into the mobile phase vs felodipine. For SFC, the explanation of retention behaviour is based on differences in CO_2 solubility between felo-

Table 3
SFC/MWD calibration curve results

	SFC/MWD only		SFC/MWD split level A†‡		SFC/MWD split level B‡‖	
	362 nm	254 nm	362 nm	254 nm	362 nm	254 nm
m*	0.39	0.47	0.48	0.57	0.56	0.67
i*	5.28	11.97	1.78	33.63	2.48	3.33
r	0.9997	0.9998	0.997	0.996	0.996	0.996
s_m	0.0013	0.0034	0.023	0.027	0.022	0.026
s_i	3.58	9.70	1.78	2.12	8.97	10.54
$s_{y/x}$	5.51	14.95	29.02	34.54	8.74	10.27
LOD (ppm)	5	12	14	14	40	56
LOQ (ppm)	18	42	47	47	133	187

SFC analysis conditions were as follows: mobile phase, 6% (v/v) methanol-modified CO_2; 25 cm × 4.6 mm i.d. Hypersil Si column; temperature, 45°C; pressure, 300 bar; flow rate, 2 ml min^{-1}; injection volume, 5 µl. Felodipine concentration was 1 mg ml^{-1}.

* Calibration curves constructed by plotting peak area vs pg felodipine injected.

† Split A = 99.6% of post-column effluent directed to the MWD.

‡ 1 µl injection used.

‖ Split B = 99.3% of post-column effluent to the MWD.

Table 4
SFC/ECD statistical [26] calibration curve* analysis

	SFC/ECD split level A†	SFC/ECD split level B‡
m*	28.6	12.9
i*	209.2	731.8
r	0.9992	0.996
s_m	585.6	537.9
s_i	327.7	1635.7
$s_{y/x}$	442.9	1652.1
LOD (pg)	34.4	381
LOQ (pg)	113	1271

SFC analysis conditions were as follows: mobile phase, 6% (v/v) methanol-modified CO_2; 25 cm × 4.6 mm i.d. Hypersil Si column; temperature, 45°C; pressure, 300 bar; flow rate, 2 ml min^{-1}; injection volume, 5 µl. Felodipine concentration was 1 mg ml^{-1}.

* Calibration curves constructed by plotting peak area vs pg felodipine injected.

† Split A = 0.4% of post-column effluent directed to the ECD.

‡ Split B = 0.7% of post-column effluent to the ECD.

dipine and H152137. It has been shown [28] that molecules that are planar have less solubility in CO_2 than do those that are non-planar. In the case of the felodipine separation, this would mean that H152/37 would elute sooner than felodipine since the pyridine ring in the former is more planar than the pyrrole ring in the latter.

Simultaneous SFC/MWD/ECD

With growing concerns over degradate identification and quantitation, the use of element selective detectors such as the ECD could be helpful in achieving accurate identification of degradates in formulated drug products as well as metabolites in biological matrices. Unfortunately, due to the flow constraints of the ECD and the presence of methanol in the SF mobile phase, the entire packed column SFC eluent could not be introduced to the ECD. As stated previously, simultaneous MWD and electron capture detection (ECD) was accomplished by splitting the flow post-column to each detector (Fig. 2). Split ratios were determined by measuring the resultant gas flows exiting each detector. An ECD temperature of 100°C was used as per manufacturer's specifications. Higher temperatures (375°C) were investigated in order to attain maximal sensitivity [29] but were found to cause severe band broadening and increased analyte retention time.

Under the conditions given in Fig. 3, the LOD of the SFC/ECD system was found to be 34.4 pg felodipine by the propagation of error method [28] (Table 4). The linear dynamic range (LDR) of the ECD under those conditions was found to be approximately one order of magnitude (r = 0.9992). Since the response of the ECD is governed by an exponential function [30], a small LDR is expected. Injection precision (n = 5) based on peak area was found to be 2.0%.

The influence of split ratio on ECD response was found to be significant in ECD performance (Table 4). As the split ratio was increased from 0.4% of the chromatographic effluent (4.5 ml min^{-1} gas to the ECD) to 0.7% (9.25 ml min^{-1} gas), the ECD was found

to be an order of magnitude less sensitive with the higher split flow ratio. The increase in split flow ratio was accomplished by using a faster flow rate integral restrictor. Three possible causes are proposed for this drastic decrease in ECD sensitivity. First, the amount of methanol entering the ECD under these conditions was doubled with the increase in split ratio. Since methanol is an electron capturing species, its increased flux to the detector could significantly increase the background signal thereby decreasing overall ECD sensitivity. The same is true for CO_2 since it has also been shown to be an electron scavenging species [29]. Secondly, the amount of P-5 gas (auxiliary ECD gas for generating thermal electrons) was not increased when the split ratio was increased. The increased amount of CO_2 and methanol introduced into the detector under the higher split flow ratio is likely to reduce sensitivity, under a constant auxiliary gas flow rate, since the number of thermal electrons available to felodipine will be reduced. Third, since the ECD was being operated at a relatively low temperature (compared to GC/ECD operation and previous SFC/ECD work [29]), the sensitivity may be more influenced by the greater Joule–Thompson cooling experienced as higher SF mobile phase flows decompress into the detector. Further research is currently being conducted in order to fully optimize ECD sensitivity under methanol-modified mobile phase conditions.

The post-column split served to decrease the linearity of the MWD response as evidenced by the lower correlation coefficient ($r = 0.996$) and the increase in s_m (Table 3). MWD sensitivity was also found to decrease significantly with the larger split ratio. These results may suggest that discrimination is occurring as a result of the split flow or that the split causes more detector noise for both detectors.

SFC/ECD/MWD traces of the drug (Peak 2) and its oxidative degradation product (Peak 1) are given in Fig. 4. The peak obtained represented the introduction of 4 ng of felodipine into the ECD based on the split ratio. A slight difference in MWD and ECD retention time was observed due to the amount of dead volume introduced by the post-column split.

SFC vs LC: analysis of a sustained-release felodipine tablet

In order to directly compare packed column SFC and analytical scale LC, 5 mg potency

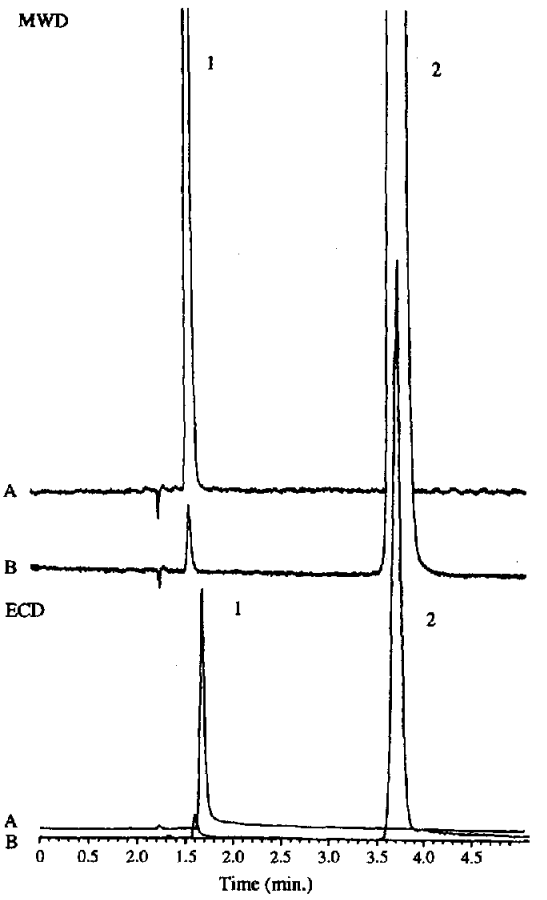

Figure 4
SFC/ECD/UV felodipine separation. SFC conditions given in Fig. 3. ECD conditions are as follows: 0.4% split to ECD; 100°C detector temperature; 400 ml min^{-1} P-10 gas flow rate. Peak identification is as follows: (1) H152/37 and (2) felodipine. Chromatograms labelled (A) represent the analysis of an H152/37 solution prepared from reference material. Chromatograms labelled (B) represent the analysis of a felodipine solution (methanol) stressed at 80°C overnight.

sustained-release felodipine tablets were analysed by SFC/MWD and LC/UV using the methods described previously. Instead of using a traditional liquid solvent extraction for sample preparation, supercritical fluid extraction (SFE) was used to remove the drug from the crushed 5 mg potency tablet. The full optimization of this SFE method is discussed elsewhere [31]. Alternatively, the tablet could have been disintegrated in methanol (in order to satisfy SFC injection solvent requirements). SFE sample preparation was chosen in order to further reduce solvent usage and disposal costs since it, like SFC, uses carbon dioxide-based fluid. In addition, the extracts produced are compatible with SFC and HPLC. The tablets ($N = 2$) were extracted using the conditions

given in the experimental section. Each rinse vial was assayed separately by both SFC/MWD and LC/UV. For SFC analysis, a 5 μl injection was employed while 20 μl were used for LC analysis. Since 20 μl is a relatively small injection volume for LC, the methanol extracts were injected as is without being diluted with water. Peak shape was found to be satisfactory under these conditions.

SFC/MWD chromatograms of several tablet extracts are given in Fig. 5. Each chromatogram represents the amount of felodipine extracted per given extraction step. No excipient interference was observed with either assay. No peak corresponding to H152/37 was also detected. Recoveries of felodipine from two crushed sustained-release tablets were 96 and 97% claim (4.80 and 4.85 mg per tablet, respectively).

Figure 6 graphically compares LC vs SFC quantitation on a vial-for-vial basis for the analysis of the SFE extract of the former tablet. As described previously, there were two methanol rinses (collected separately) for each of the four extraction steps, making a total of eight extract solution vials. Felodipine recoveries of 96.0% claim (SFC extract analysis) and 96.6% claim (LC analysis) were obtained. As can be seen from the plot, good agreement was obtained regardless of the amount of felodipine present in the individual fractions. All differences in % claim were within experimental error.

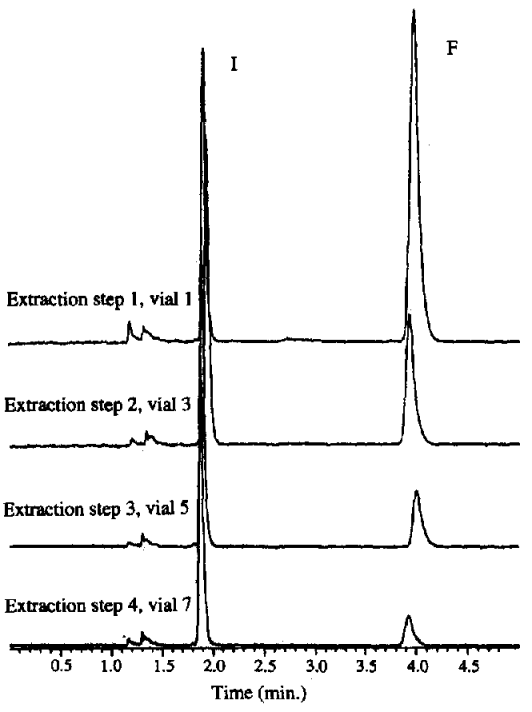

Figure 5
SFC/UV felodipine tablet analysis. SFC conditions are as in Fig. 3. Conditions for SPE sample preparation given in the text. Each chromatogram represents the analysis of the indicated SFE trap rinse vial. Peak identification is as follows: (I) internal standard (carbazole) and (F) felodipine.

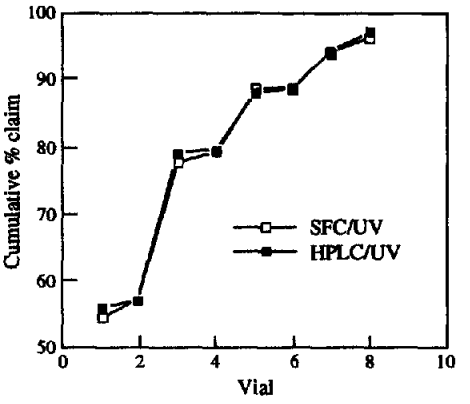

Figure 6
Comparison of SFC/UV vs LC/UV tablet analysis. SFC conditions are as in Fig. 3, LC conditions are given in the Experimental section. SFE used to prepare tablet prior to chromatographic analysis.

The equivalency in analysis became particularly striking when the solvent usage and sample throughput for SFC vs LC was compared (Table 5). When the SFC/MWD system was used for analysis, sample throughput was increased by 60% over an analogous LC separation. Although more total mobile phase was used for SFC, only 6% of the SFC mobile phase is actually disposable solvent waste. The remaining 94% is carbon dioxide gas which was vented to a hood.

The disposal cost of 100% organic solvent (non-chlorinated) vs water/organic solvent mixtures also illustrates another advantage of the SFC assay. The most common procedure for solvent waste disposal is combustion in large scale manufacturing furnaces. Such furnaces typically combust 45,000 gallons of solvent waste per hour. Since water/organic mixtures, generated from LC analysis, produce less heat (<3000 B.t.u. lb^{-1}) upon combustion, the resulting cost of disposal to the waste source is higher. Conversely, 100% organic solvent disposal (generated by SFC) has a higher fuel value (9500 B.t.u. lb^{-1}) and, therefore, its cost of disposal is less.

Table 5
Solvent usage comparison: packed column SFC vs LC

	Packed column SFC/UV	Analytical scale LC/UV
Mobile phase	6% (v/v) methanol-modified CO_2	Acetonitrile–methanol–50 mM phosphate buffer (pH 3) (40:20:40, v/v/v)
Samples analysed h^{-1}	10 (6 min, run time)	4 (15 min run time)
ml Mobile phase used h^{-1}	120	90
ml Disposable waste h^{-1}	7.2	90
Mobile phase disposal cost per 55 gal.*	$48†	$175†

*See text for disposal description.
†Disposal cost obtained from Solid Waste Management, Merck Research Laboratories, West Point, PA, USA.

Conclusions

Packed column SFC was shown to be a viable chromatographic technique for the analysis of pure felodipine drug as well as felodipine in sustained-release tablets. The SFC method was shown to be reproducible and selective for the separation of felodipine from its oxidative degradation product, H152/37, by the use of 6% (v/v) methanol-modified CO_2 at 280 bar and 45°C. The resolution of the degradate from the parent compound was found to be less influenced by separation conditions than that obtained in HPLC. With the equipment used, simultaneous SFC/ECD/MWD of felodipine was demonstrated. The combination of SFC with gas phase detectors represents a unique means of: (1) identifying metabolite or degradation products of thermally labile analytes; (2) minimizing the production of aqueous/organic disposable waste; and (3) decreasing overall organic solvent usage. Since ECD with methanol-modified CO_2 mobile phase has not been used extensively, further optimization is required for it to reach its full potential. The LOD and LOQ for felodipine under SFC conditions were found to be slightly higher than that for LC as a result of the smaller injection volumes employed.

SFE was shown to be a viable alternative to solvent extraction sample preparation. Its use will be advantageous in cutting solvent usage for sample preparation. However, further optimization of extraction conditions are necessary in order to improve felodipine recoveries. The use of SFC/MWD for tablet analysis (vs HPLC/UV) was shown to be more cost effective from both the organic solvent usage and disposal standpoints. Even the use of 2 mm columns in HPLC/UV analysis (assuming ~50% waste reduction vs 4.6 mm i.d. columns) do not provide the solvent savings seen for SFC/UV analysis. In addition, sample throughput was increased by 60% with the SFC/MWD assay.

Acknowledgements — The authors would like to thank Hewlett-Packard for the use of both the SFE (Model 7680T) and SFC equipment. The authors would like to acknowledge the SFC technical support of Dr Richard Kornfeld (HP). Lastly, the authors would like to acknowledge Merck and Co., Inc. for the research grant to Virginia Polytechnic Institute and State University which made this work possible.

References

[1] S. Higuchi, H. Sasaki and T. Sado, *J. Chromatogr.* **110**, 301–307 (1975).
[2] P. Jacobsen, O. Lederballe Pedersen and E. Mikkelsen, *J. Chromatogr.* **162**, 81–87 (1979).
[3] S. Kondo, A. Kuchiki, K. Yamamoto, K. Akimoto, K. Takahashi, N. Awata and I. Sugimoto, *Chem. Pharm. Bull.* **28**, 1–7 (1980).
[4] J. Dokladalova, J.A. Tykal, S.J. Coco, P.E. Durkee, G.T. Quercia and J.J. Korst, *J. Chromatogr.* **231**, 451–458 (1982).
[5] S.R. Hamann and R.G. McAllister, Jr, *Clin. Chem.* **29**, 158–160 (1983).
[6] L.J. Lesko, A.K. Miller, R.L. Yeagerand, D.C. Chatterji, *J. Chromatogr. Sci.* **21**, 415–419 (1983).
[7] M. Ahnoff, *J. Pharm. Biomed. Anal.* **2**, 519–526 (1984).
[8] R. Nishioka, I. Umeda, N. Oi, S. Tabata and K. Uno, *J. Chromatogr.* **565**, 237–246 (1991).
[9] P.A. Soons, M.C.M. Roosemalen and D.D. Breimer, *J. Chromatogr.* **528** (1990).
[10] M.T. Rossel and G. Bogaert, *J. Chromatogr.* **279**, 675–680 (1983).
[11] S. Higuchi and Y. Shiobara, *Biomed. Mass Spectrom.* **5**, 220–223 (1978).
[12] S. Higuchi and Y. Shiobara, *Biomed. Mass Spectrom.* **7**, 339–344 (1980).
[13] S. Higuchi and S. Kawamura, *J. Chromatogr.* **223**, 341–349 (1981).
[14] R. Testa, E. Dolfini, C. Reschiotto, C. Secchi and P.A. Biodi, *Farmaco Ed. Prat.* **34**, 463–473 (1979).
[15] M. Gabrielsson, K.-J. Hoffmann and C. Gunnar Regårdh, *J. Chromatogr.* **573**, 265–274 (1992).
[16] O. Gyllenhaal and J. Vessman, *J. Chromatogr.* **628**, 275–281 (1993).

[17] M. Masuda, S. Koike, M. Handa, K. Sagara and T. Mizutani, *Anal. Sci.* **9**, 29–32 (1993).
[18] S. Scalia and D.E. Games, *J. Pharm. Sci.* **82**, 44–47 (1993).
[19] N.K. Jagota and J.T. Stewart, *J. Liq. Chromatogr.* **16**, 291–305 (1993).
[20] N.K. Jagota and J.T. Stewart, *J. Liq. Chromatogr.* **15**, 2429–2443 (1992).
[21] N.K. Jagota and J.T. Stewart, *J. Chromatogr.* **604**, 255–260 (1992).
[22] J.R. Perkins, D.E. Games, J.R. Startin and J. Gilbert, *J. Chromatogr.* **540**, 239–256 (1991).
[23] E.D. Ramsey, J.R. Perkins, D.E. Games and J.R. Startin, *J. Chromatogr.* **464**, 353–364 (1989).
[24] J.L. Janicot, M. Caude and R. Rosset, *J. Chromatogr.* **437**, 351–364 (1988).
[25] Method 'HPLC Analysis of Felodipine in Sustained-Release Tablets' published internally at AB Hassle, *Analytical Chemistry*, **Fer 6-1**, pp. 6–11.
[26] C. Båårnhielm, I. Skanberg and K.O. Borg, *Xenobiotica* **14**, 719–726 (1984).
[27] J.D. Winefordner and G.L. Long, *Anal. Chem.* **55**, 712–742A (1983).
[28] G.S. Gurdal and N.R. Foster, *Ind. Eng. Chem. Res.* **30**, 575–580 (1991).
[29] H.-C.K. Chang and L.T. Taylor, *J. Chromatogr. Sci.* **28**, 29–35 (1990).
[30] A. Zlatkis and C. Poole (Eds), *Electron Capture: Theory and Practice in Chromatography*, Vol. 20, Chapter 1. Elsevier, Amsterdam (1982).
[31] A.L. Howard, D.C. Shah, D. Ip, M.A. Brooks, L.T. Taylor and J.T.B. Strode III, in press.

[Received for review 9 December 1993; revised manuscript received 22 February 1994]