ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1513-1520

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Supercritical fluid extraction–liquid chromatography method development for a polymeric controlled-release drug formulation

Daryl A. Roston [a,*], Jeffrey J. Sun [a], Paul W. Collins [a], William E. Perkins [a], Samuel J. Tremont [b]

[a] *Searle Research and Development, 4901 Searle Parkway, Skokie, Illinois 60077, USA*
[b] *Monsanto Corporate Research, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, USA*

Received for review 6 December 1994; revised manuscript received 28 April 1995

Abstract

We have recently been involved in the development of a method for assaying the active component in a controlled-release drug formulation, which is composed of a drug substance covalently bonded to polymer matrix. The drug substance in the formulation is the active enantiomer of misoprostol, a synthetic analog of natural prostaglandins and the active ingredient in Cytotec®. Our method development consisted of a systematic evaluation of dynamic, off-line supercritical fluid extraction (SFE) as sample preparation for the formulation assay. Extracts were analyzed with normal phase and reversed-phase HPLC methods. The reversed-phase system utilized postcolumn reaction to provide selective detection of the extracted prostaglandin sample components. Several SFE parameters were investigated to optimize the recovery of the drug substance from the formulation, including sample quantity, extraction cell volume, extraction duration, supercritical carbon dioxide modifier, temperature, pressure, and collection solvent. The SFE experiments were completed with a commercially available multicell extractor. Preliminary validation studies utilized a formulation made with radiolabeled drug to determine the recovery achieved under the optimized SFE conditions and assessed the precision of replicate determinations. Analysis was completed under the optimized conditions to quantitate levels of the active component and related compounds in lots of the experimental polymeric formulation and to determine the total weight per cent extracted.

Keywords: Supercritical fluid extraction; Liquid chromatography; Polymeric formulation

1. Introduction

We have recently been involved in the development of an assay for an experimental, controlled-release drug formulation, which consisted of the drug substance (active ingredient) covalently linked to a polymer. The drug substance in the formulation is the active enantiomer of misoprostol (misoprostol-AE), a synthetic analog of natural prostaglandin E_1 and the active ingredient in Cytotec®, which is used for the prevention of non-steroidal anti-inflammatory drug induced ulcers. The experimental formulation consisted of misoprostol-AE covalently linked to a polybutadiene polymer, which was cross-linked after the covalent linkage. Structures for misoprostol-AE and the experimental polymeric formulation (EPF) are depicted in Fig. 1 (top). The EPF was a controlled-release formulation designed to minimize drug side-effects. As shown in Fig. 1, misoprostol-AE was linked to the polymer via a silyl ether bond to its C-11 hydroxy group. An important element of the EPF was the pH

* Corresponding author.

SSDI 0731-7085(95)01588-4

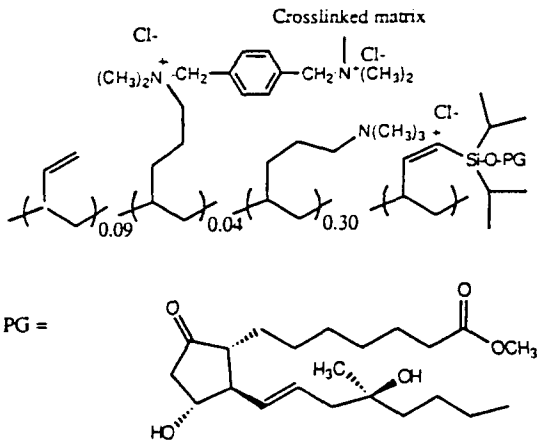

Fig. 1. Misoprostol-AE and EPF structures.

sensitivity of the silyl ether covalent bond, which resulted in a controlled release of the misoprostol-AE in the acidic conditions in the stomach, but not in the neutral or alkaline intestinal environment, therefore minimizing unwanted intestinal side-effects. More detailed descriptions of the rationale behind the development of the EPF, EPF release profiles, and the EPF synthesis have been reported elsewhere [1,2].

The potential use of EPF in toxicological studies and clinical trials stimulated the development of (an) analytical method(s) that could be used to assay EPF lots for misoprostol-AE and related compounds. Analytical method development for the EPF posed a considerable challenge for several reasons.

(1) The thermal and chemical lability of misoprostol-AE limited the rigor of the extraction conditions that could be used to recover the undegraded active ingredient.

(2) Since the analyte was covalently linked to the polymer matrix, the extraction solvent had to effect a cleavage of the covalent linkage of the misoprostol-AE polymer before separation of the analyte from the matrix.

(3) Covalent linkage of the analyte also diminished the utility of conventional "spiking" experiments for recovery determinations.

(4) The cross-linking of the polymer matrix yielded samples with an extremely high molecular weight and rendered them insoluble in virtually any solvent.

(5) The cross-linking also rendered the EPF samples less penetrable by liquid extraction solvents and limited the applicability of liquid–solid extractions for yielding quantitative recovery of misoprostol-AE and related compounds.

The unique elements and difficulty of the analytical method development for the EPF led us to investigate the use of supercritical fluid extraction (SFE) as a sample preparation procedure for EPF lot analysis. SFE is gaining widespread popularity as a sample preparation procedure for diverse types of samples, including environmental [3,4], agricultural-food [5–7], and pharmaceutical samples [8–11]. Recent reports have shown that SFE is a "flexible" methodology that involves complex physical-chemical phenomena [3,12–14] and that SFE method development requires careful optimization of several parameters [15–18] (the cited references represent an extensive field). The present study concerns the development and optimization of an SFE–HPLC procedure for the determination of the misoprostol-AE/prostaglandin content of EPF samples. Supercritical fluid extracts of various EPF samples were analyzed with normal phase and reversed-phase HPLC procedures. The reversed-phase system utilized postcolumn reaction to provide selective, sensitive detection of the extracted prostaglandin sample components. Development studies for the SFE procedure involved optimization of the collection solvent, system pressure, extraction time, and the modifier for the carbon dioxide extraction media. An important part of the method development was the discovery that supercritical carbon dioxide modified with 5% formic acid was an effective extraction media for cleaving the covalent linkage between misoprostol-AE and the polymer, and yielded high recoveries of the prostaglandin content of EPF samples in a short extraction period. Prostaglandin recoveries with the optimized conditions were estimated with the use of an EPF sample synthesized with radiolabeled misoprostol-AE. Lot analysis for the EPF samples estimated total prostaglandin levels of 1–2 parts per thousand (ppt) for several research lots. The weight per cent of the EPF samples extracted during SFE ranged from 2.8 to 3.6%.

2. Experimental

2.1. Materials and reagents

EPF lots were synthesized by Monsanto Corporate Research or by the Chemical Sciences Department of Searle Research and Development. Standards for misoprostol-AE and

related prostaglandin (PG) compounds were provided by the Chemical Sciences Department. Average width and length particle size ranges for eight EPF lost were as follows: width, 10–172 μm; length, 33–291 μm. EPF samples available during the initial method development studies were mixed (1:1) with hydroxyproplymethycellulose (HPMC).

All HPLC mobile phase components were reagent-grade and were used as purchased. All SFE experiments were completed with supercritical fluid grade carbon dioxide purchased from Scott Specialty Gases. Carbon dioxide modifiers for SFE experiments were administered with premixed cylinders.

2.2. SFE experiments

All SFE experiments were completed with a Dionex model 703 supercritical fluid extractor. The model 703 extractor is an eight-channel extractor; however, simultaneous use of three cells was typically employed. Experiments performed at pressures below 340 atm (5000 psi) were implemented with cells purchased from Dionex. Experiments performed at pressures above 340 atm were completed with cells purchased from Keystone Scientific.

The SFE conditions for EPF lot analyses were as follows: extractant, carbon dioxide with 5% formic acid (w/w); pressure, 330 atm; oven temperature, 75°C; cell volume, 0.5 ml; sample quantity, 25 mg; restrictor temperature, 100°C; collection solvent, hexane–ethanol (2:1, v/v); collection solvent volume, 15 ml; collection solvent temperature, 0°C; extraction time, 60 min. Approximately 25 mg of the EPF sample were placed directly in the extraction cell. After completion of the SFE experiment, the collection solvent was evaporated to dryness under nitrogen and redissolved in 1 ml of mobile phase before injection into the HPLC.

2.3. HPLC system

All HPLC experiments were completed with a component system comprised of a Hewlett Packard 79855A autosampler, a Hewlett Packard 79852A pumping system, and a Kratos 783 variable wavelength detector. Postcolumn reagent was administered with a Kratos Model URS501 pump and a "t" connector between the column outlet and detector inlet. Initial SFE experiments were analyzed with a normal phase HPLC system, using the following conditions: column, Zorbax Si (250 mm × 4.6 mm i.d.); mobile phase, dioxane–isoctane–acetonitrile (21.5:78.0:0.5, v/v/v); flow rate, 2.0 ml min^{-1}; injection volume, 10 μl; detection, 205 nm. Reversed-phase HPLC conditions were as follows: column, Supelco ODS (250 mm × 4.6 mm i.d.); mobile phase, acetonitrile–methanol–water (45:20:35, v/v/v); flow rate, 1.5 ml min^{-1}; injection volume, 10 μl; postcolumn reagent, 4 M KOH; postcolumn reagent flow rate, 0.5 ml min^{-1}; postcolumn temperature, 80°C; detection wavelength, 280 nm. Quantitation of the prostaglandin levels in the samples were completed by comparison of the HPLC peak areas for the extract components and standards of misoprostol-AE and related compounds.

2.4. Radiolabeling experiments

Extraction recovery was estimated with an EPF sample synthesized with a radiolabeled (tritium) misoprostol sample. Specific activity assays for EPF samples were performed with a Packard model 307 Oximate 80 oxidizer followed by liquid scintillation counting for the trapped volatile products. Liquid scintillation counting was performed with a Packard Tri-Carb 2000 CA liquid scintillation analyzer. Recovery estimates were based on normalizing the tritium concentration for the SFE collection solvent to the tritium concentration for the unextracted EPF sample.

3. Results and discussion

3.1. Preliminary SFE–HPLC experiments

The results of preliminary SFE–HPLC experiments for the analysis of EPF samples are shown in Figs. 2A–2F. The chromatograms shown in Fig. 2 were generated under normal phase conditions and with UV detection. Figs. 2A and 2B are the result of 30 min extractions of two EPF samples, which were synthesized with different concentrations of misoprostol-AE to yield different levels in the EPF samples ("high-load" and "low-load"). The extractions corresponding to Figs. 2A and 2B were carried out with carbon dioxide modified with 5% formic acid. Comparison with Fig. 2G, which is a chromatogram of a misoprostol-AE standard, shows that the use of formic-acid-modified carbon dioxide affected the liberation

of misoprostol-AE from the EPF samples. Experiments completed under the same conditions except that the modifier was 5% methanol (Figs. 2D and 2E) did not generate detectable levels of misoprostol-AE. Extractions of an EPF sample without covalently linked misoprostol-AE (polymer blank) are shown in Figs. 2C and 2F, which were generated with formic acid- and methanol-modified carbon dioxide, respectively.

The results represented in Fig. 2 were promising and led to more extensive method development studies. It was evident from the complexity of the chromatograms in Fig. 2 that the SFE conditions were extracting polymer components as well as misoprostol-AE. Since method development studies were focused towards a procedure that could assay the misoprostal-AE content of the EPF samples, we changed the HPLC conditions to yield selectivity for the prostaglandin components. Figs. 3A and 3B show chromatograms of EPF extracts, which were analyzed with a reversed-phase HPLC system and postcolumn reaction detection. The postcolumn reagent was 4 M KOH, which converted eluting prostaglandins to a base degradation product ("B-from"; note structures in Fig. 3) that has an absorbance maximum at 280 nm. Fig. 3B is a chromatogram of a five-component mixture containing misoprostol-AE and four related compounds. Fig. 3A is a chromatogram of a supercritical fluid extract of an EPF sample. It is evident from the chromatograms in Fig. 3 that the postcolumn system provided selectivity for the prostaglandin components of the EPF extracts. The chromatogram represented in Fig. 3A is a typical supercritical fluid extract of an EPF sample, with major components being misoprostol-AE and the acid degradation product ("A-form"; note structures in Fig. 3). Prostaglandin impurity levels in the SFE extracts were the same as the levels observed in the drug substance lots prior to coupling to the polymer, except for the relatively high A-form levels.

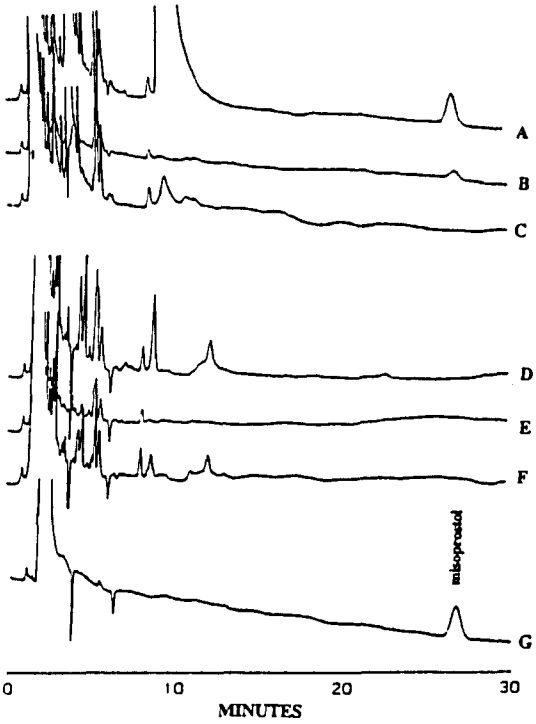

Fig. 2. Normal phase HPLC chromatograms of SFE extracts of an EPF sample: (A) high-load EPF extract, 5% formic acid modifier; (B) low-load EPF extract, 5% formic acid modifier; (C) EPF "polymer blank" extract, 5% formic acid modifier; (D) high-load EPF extract, 5% methanol modifier; (E) low-load EPF extract, 5% methanol modifier; (F) EPF "polymer blank" extract, 5% methanol modifier; (G) misoprostol-AE standard. Chromatography conditions: column, Zorbax Si (250 mm × 4.6 mm i.d.); mobile phase, dioxane–isoctane–acetonitrile (21.5:78.0:0.5, v/v/v); flow rate, 2.0 ml min^{-1}; injection volume, 10 μl; detection wavelength 205 nm.

3.2. SFE optimization studies

The reversed-phase HPLC system with postcolumn reaction detection utilized to generate the chromatograms in Fig. 3 provided a method for monitoring the effect of changes in the SFE parameters on the prostaglandin recoveries. Our subsequent work centered on optimizing the SFE conditions for the EPF/prostaglandin analysis. Efforts centered on the effect of changes in seven SFE parameters on the prostaglandin recovery: collection solvent, collection solvent volume, carbon dioxide modifier, pressure, cell size, sample quantity, and extraction duration. Results of the optimization studies are expressed in Tables 1–3 as parts per thousand (ppt) prostaglandin and are the sum of the misoprostol-AE and related compounds recovered during the various experiments. Due to sample availability during early project stages, EPF samples as well as EPF mixed 1:1 with HPMC (EPF:HPMC) were used during the optimization experiments. The intention of the optimization study was to determine which SFE parameters could be exploited to maximize the recovery of the misoprostol-AE/prostaglandin content of the EPF samples.

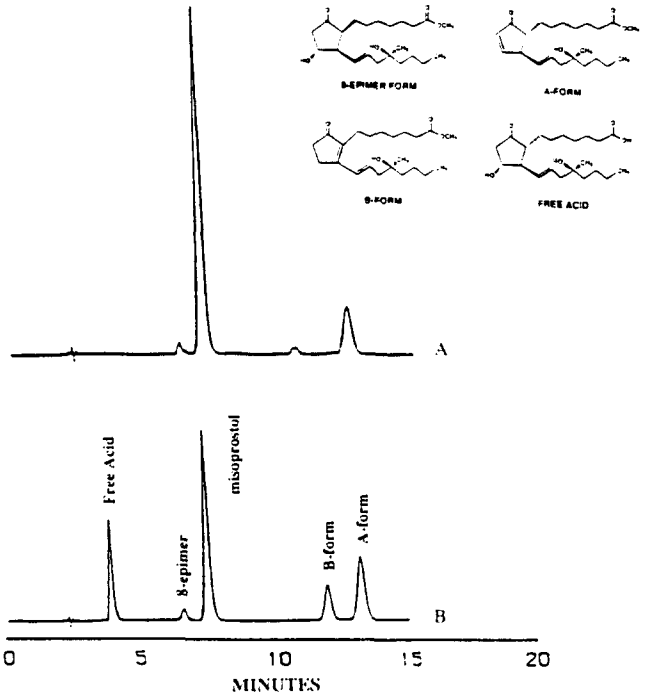

Fig. 3. Reversed-phase HPLC chromatograms of SFE extracts of an EPF sample and misoprostol-AE standards: (A) EPF extract; (B) misoprostol-AE standards. Chromatography conditions: column, Supelco ODS (250 mm × 4.6 mm i.d.); mobile phase, acetonitrile–methanol–water (45:20:35, v/v/v); flow rate, 1.5 ml min^{-1}; injection volume, 10 μl; postcolumn reagent, 4 M KOH; postcolumn reagent flow rate, 0.5 ml min^{-1}; postcolumn temperature, 80°C; detection wavelength 280 nm.

Collection solvent

Since the outcome of all other optimization experiments is dependent on the collection efficiency of the SFE system, one of the first parameters that we studied was the collection solvent for the extraction system. Results from the collection solvent studies with EPF:HPMC samples are summarized in Table 1A and assess the effect of the type of solvent and the solvent volume on the total prostaglandin recovery during 30 min extractions. The data in Table 1A represent two sets of experiments, with six extractions being performed in parallel during each set of experiments. When six solvents were evaluated during single extractions completed in parallel, the highest recoveries were observed with a hexane–ethanol mixture. Also, the recovery increased as the volume of the solvent was increased from 2.5 to 15 ml, with a maximum value for of 1.2 ppt for an EPF:HPMC sample. Solvent volumes greater than 15 ml resulted in low recoveries due to loss of solvent and analyte, caused by the decompression and bubbling of the extraction media.

Cell size and sample quantity

Initial SFE experiments wtih EPF samples were implemented with 0.5 ml extraction cells and 25 mg of sample. We investigated the use of larger and smaller cell volumes and various sample quantities to learn if different combinations of cell volumes and sample quantities resulted in greater analyte recoveries. As summarized in Table 1B, four different cell sizes, ranging from 0.17 to 10.0 ml, were investigated using EPF:HPMC samples. The highest recovery values of 1.1 ppt were obtained with the 0.5 ml cell volume, using 25 mg of sample. Use of larger or smaller cell volumes or larger sample quantities generally reduced the recovery of the prostaglandins during a 30 min extraction. It should be noted that 25 mg of EPF sample filled only a fraction of the volume of the 0.5 ml cell. During the extraction, the sample swelled significantly. Sample swelling has previously been correlated with analyte extractability in a study reported by McNally and co-workers [14].

Pressure and carbon dioxide modifier

Additional optimization studies, which concerned the system pressure and carbon dioxide modifier, are summarized in Table 2A. The 30 min extractions were completed at 280, 330 and 600 atm, which represents an approximate supercritical fluid density range of 0.74–

0.93 g ml^{-1}. A possible explanation for the observed pressure–recovery relationship is that recovery increases with the linear velocity of the extraction media through the cell up to a certain velocity, and then begins to decrease. The highest recoveries of 1.8 ppt prostaglandin for an EPF sample were observed at the intermediate pressure of 330 atm. When the formic acid modifier level was changed from 5% to 1%, the recovery for a 30 min extraction decreased from 1.8 to 1.0 ppt. Also, an extended extraction (150 min) was completed using carbon dioxide with 5% methanol as a modifier rather than 5% formic acid. The prostaglandin recovery for the extended duration experiment was quite low at 0.1 ppt.

Extraction duration

We also studied the effect of extraction duration on the prostaglandin recovery, by performing experiments of duration 30, 45 and 60 min, as well as two sequential 35 min experiments. Results summarized in Table 2B, which were generated with EPF samples, show that increasing the duration of the extraction within the time periods evaluated did not produce an apparent increase in the prostaglandin recovery, which was found to be 1.8 ppt. To gain a better understanding of the absolute prostaglandin recovery–extraction duration relationship, experiments were completed with an EPF sample synthesized with radiolabeled misoprostol. Recovery was calculated by normalizing the tritium concentration in the collection solvent after 75 min of extraction to the tritium concentration in the original sample. Results are summarized in Table 3 and show that 85% of the prostaglandin content was recovered during the initial 45 min of the extraction. An additional 5% was recovered during the subsequent 30 min to bring the total recovery to 90%. The varying duration and

Table 1
Collection solvent, sample quantity, cell volume[a]
A

PG level (ppt)	Collection solvent	Solvent vol. (ml)
1.2	Hexane–ethanol	15
0.4	Hexane–ethanol	10
0.4	Hexane–ethanol	7.5
0.4	Hexane–ethanol	7.5
0.4	Hexane–ethanol	2.5
0.6	Hexane–ethanol	10
0.5	Acetonitrile	10
0.3	Hexane	10
0.2	Ethanol	10
0.2	Acetone	10
0.0	Ethyl acetate	10

B

PG level (ppt)	Sample quantity (mg)	Cell vol. (ml)
0.6	25	0.17
1.1	25	0.5
0.9	100	0.5
0.2	200	3.5
0.4	500	3.5
0.3	50	10
0.2	100	10
0.4	500	10

[a] Experiments completed with EPF:HPMC samples; $n = 1$ for each determination.

Table 2
Pressure, carbon dioxide modifier, duration
A

PG level (ppt)	n	RSD	Extraction time (min)	Modifier	Pressure (atm)
1.6	3	10.2	30	5% FOR	280
1.8	3	12.0	30	5% FOR	330
1.3	3	22.9	30	5% FOR	600
1.0	3	5.3	45	1% FOR	330
0.1[a]	1	–	150	5% MEOH	330

B

PG level (ppt)	n	rsd	Extraction time (min)	Modifier	Pressure (atm)
1.8	3	4.6	30	5%FOR	330
1.8	3	12.0	45	5%FOR	330
1.8	3	3.2	60	5%FOR	330
1.9	3	7.6	35(×2)	5%FOR	330

[a] Experiment completed with EPF:HPMC.
Key: FOR, formic acid; MEOH, methanol.

Table 3
Radiolabeled sample study; per cent extracted

	Per cent extracted			Per cent remaining on polymer	Per cent in waste
	Total	First 45 min	Subsequent 30 min		
$n = 3$	90	85	5	4	< 1
S.D.	4.0	4.5	5.9	1.2	–

radiolabeling studies indicated that most of the prostaglandin was recovered during the first 30 min of extraction, with smaller portions being recovered during subsequent 30–45 min periods.

Other SFE parameters

Several SFE parameters besides those investigated in the experiments represented in Tables 1–3 are important factors for the recovery; however thorough optimization studies for these parameter were not completed due to sample stability or instrument design limitations. These include the volume flow rate, oven temperature, restrictor temperature, and collection solvent temperature. With the Dionex model 703 supercritical fluid extractor, the volume flow rate is set with prefabricated restrictors that are set at 250 or 500 ml min^{-1}. The 30 min extractions of EPF samples completed with the 250 and 500 ml min^{-1} restrictors did not yield different prostaglandin recoveries. The collection vial temperature for all experiments was 0°C, which was the lowest temperature obtainable with the Dionex model 703 supercritical fluid extractor. Thorough system temperature studies were not completed since the thermal lability of prostaglandins precluded the use of higher oven and restrictor temperatures. All experiments were completed with oven and restrictor temperatures of 75°C and 100°C, respectively.

3.3. EPF lot analysis

The results expressed in Tables 1–3 demonstrate that each of the studied experimental parameters exerted an influence on the prostaglandin recovery. The following optimized parameters were used to conduct a preliminary analysis of several EPF process development lots: collection solvent, hexane–ethanol (2:1); collection solvent volume, 15 ml; cell volume, 0.5 ml; sample quantity, 25 mg; carbon dioxide modifier, 5% formic acid; system pressure, 330 atm; extraction duration, 60 min.

Data summarized in Table 4 show that prostaglandin levels for several EPF synthetic process development lots ranged from 1.4 to 1.9 ppt. The average relative standard deviation (RSD) for the six analyses summarized in Table 1, which were completed during 3 days, was 6.4%. Prostaglandin levels in Table 4 are mean values for simultaneous duplicate or triplicate determinations and are not corrected for a less than 100% recovery. All the analyses were initiated as triplicate determinations; however, restrictor clogging occurred during several of the analyses and stopped the flow of the extraction media through the cell and was considered a determinate error for the analysis in the affected cell. Restictor clogging was readily evident via the channel flow rate readout of the Dionex model 703 extractor. It should be noted that analyses of EPF samples were attempted with liquid–solid extraction conditions using hexane with 5% formic acid of 5% acetic acid. The 3 h extractions generated prostaglandin levels that were below 0.1 ppt.

Table 4
EPF lot analysis

Lot	PG level (PPT)	RSD	Day	*n*	Weight per cent extracted
1	1.4	5.2	1	2	3.1
2	1.7	4.5	2	2	3.3
3	1.7	3.4	3	2	3.2
4	1.9	4.8	3	3	2.8
5	1.5	11.7	1	3	–
6	1.6	8.6	2	3	3.6

An additional analytical parameter for the EPF lots that was estimated concurrently with the prostaglandin levels was the weight per cent extracted. Weight per cent extracted values are also summarized in Table 4 and ranged from 2.8 to 3.6%. These values were obtained by tarring the collection vial before the extraction experiment and weighing the vial and extraction residue after the solvent evaporation to determine the total quantity of EPF sample extracted. The weight per cent data showed that significantly high levels of polymeric sample components were being coextracted with the analytes.

4. Conclusions

Investigations portrayed in the present report are preliminary; however, they demonstrate that SFE can be a powerful tool for addressing challenging sample preparation and analysis problems. The zero surface tension of the supercritical fluid was undoubtedly an important extraction media property, with regard to penetration of the EPF matrix. More thorough validation studies would be required

prior to acceptance of the method for routine GLP testing. Important elements of a more thoroughly validated procedure would include the use of a radiolabeled standard for correction for less than 100% recovery and sample particle size specifications. Future studies would also involve identification of the polymer coextractants.

Acknowledgments

The EPF project was a collaborative project between Monsanto Corporate Research, Searle Discovery Research, the Searle Chemical Sciences Department, and the Searle Pharmaceutical and Analytical Sciences Department. The authors gratefully acknowledge the experimental contributions of John Ng of the Searle Chemical Sciences Department and Charles Markos of the Searle Pharmacokinetics, Bioanalytical, and Radiochemistry Department.

References

[1] S.J. Tremont, P.W. Collins, W.E. Perkins, R.L. Fenton, D. Forster, M.P. McGrath, G.M. Wagner, A.F. Gasiecki, R.G. Bianchi, J.J. Casler, C.M. Ponte, J.C. Stolzenbach, P.H. Jones, J.K. Gard and W.B. Wise, J. Med. Chem., 36 (1993) 3087–3097.

[2] W. Perkins, R. Bianchi, S. Tremont, P. Collins, J. Casler, R. Fenton, G. Wagner, M. McGrath, J. Stolzenbach, D. Kowalski, A. Gasieki, D. Forster and P. Jones, J. Pharmacol. Exp. Ther., 259 (1994) 151–156.

[3] S. Hawthorne, D. Miller, M. Burford, J. Langenfeld, S. Eckert-Tilotta and P. Louie, J. Chromatogr., 642 (1994) 301–317.

[4] J. Snyder, R. Grob, M. McNally and T. Oostdyk, J. Chromatogr. Sci., 31 (1993) 183–191.

[5] R. Cross, J. Ezzell and B. Richter, J. Chromatogr. Sci., 31 (1993) 162–169.

[6] A. Howard, C. Braue and L. Taylor, J. Chromatogr., Sci., 31 (1993) 323–329.

[7] R. Marsili and D. Callahan, J. Chromatogr. Sci., 31 (1993) 422–428.

[8] D. Messer, L. Taylor, W. Moore and W. Weiser, Therap. Drug Monitoring, 15 (1993) 581–587.

[9] H. Liu, L. Cooper, D. Raynie, J. Pinkston and K. Wehmeyer, Anal. Chem., 64 (1992) 802–806.

[10] L. Mulcahey and L. Taylor, Anal. Chem., 64 (1992) 981–984.

[11] J. Dean and J. Lowdon, Analyst, 118 (1993) 747–751.

[12] J. King, J. Chromatogr. Sci., 27 (1989) 355–363.

[13] J. Langnfeld, S. Hawthorne, D. Miller and J. Pawliszyn, Anal. Chem., 65 (1993) 338–344.

[14] T. Fahmy, M. Paulaitis, D. Johnson and M. McNally, Anal. Chem., 65 (1993) 1462–1469.

[15] R. Majors, LC–GC, 10 (1992) 14–19.

[16] L. Mulcahey and L. Taylor, Anal. Chem., 64 (1992) 2352–2358.

[17] R. Smith and M. Burford, J. Chromatogr., 600 (1992) 175–181.

[18] J. Langenfeld, M. Burford, S. Hawthorne and D. Miller, J. Chromatogr., 594 (1992) 297–307.