ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 421–428

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Development and validation of an HPLC assay for fentanyl, alfentanil, and sufentanil in swab samples

John Lambropoulos *, George A. Spanos, Nick V. Lazaridis

Analytical Method Development and Validation, AA1 Incorporated, 1206 North 23rd Street, Wilmington, NC 28405, USA

Received 26 January 1999; received in revised form 16 February 2000; accepted 18 February 2000

Abstract

A high performance liquid chromatography (HPLC) method for the assay of fentanyl citrate, alfentanil hydrochloride, and sufentanil citrate swab samples was developed and validated in order to control a cleaning procedure. The swabbing procedure involved Super POLX 1200 wipers moistened with water. The assay employed extraction of swabs with water and analysis by isocratic, reversed-phase, HPLC with varying ultraviolet (UV) detection for desired sensitivity, depending on the analyte. The method was shown to be selective and linear from the limits of quantitation (0. 10, 0. 20, and 0. 15 μg/swab for fentanyl citrate, alfentanil, and sufentanil, respectively) to over three times these concentrations. The assay limits (detection levels) per swab area were set at least at 0.2% of the concentrations of the actives in the drug products (0.02, 0. 10, and 0. 10 μg/swab or approximately 0.03, 0.02, and 0.2% for fentanyl citrate, alfentanil, and sufentanil, respectively). It should be noted that all active concentrations listed in this work were calculated based on the salt form concentration for fentanyl (citrate salt) and the free base forms for alfentanil and sufentanil. No reference standard was available for alfentanil hydrochloride and sufentanil citrate. Drug product was used instead throughout this study.

Keywords: Liquid chromatography; Assay; Fentanyl; Alfentanil; Sufentanil; Swabs; Cleaning

1. Introduction

Fentanyl, 1-(2-phenethyl)-4-*N*-(*N*-propionylanilino)piperidine [1–6], is an intravenous or intramuscular synthetic narcotic (opioid) analgesic widely used for the purpose of neuroleptic analgesia and sedation during preoperative, induction, maintenance, and postoperative surgical periods.

Alfentanil, *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)4-piperidinyl]-*N*-propanamide [5–7], is a narcotic analgesic, structurally similar to fentanyl and has about one third the (clinical) potency of fentanyl. It is recommended as an alternative when used with inhalational oxygen via incremental IV bolus or continuous IV infusion in general surgery lasting less than 2 h. Sufentanil, *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide [6,8] is a very potent narcotic analgesic from the same family of sedatives,

* Corresponding author. Tel.: +1-919-4935718.

0731-7085/00/$ - see front matter
PII: S0731-7085(00)00312-5

used to treat patients with severe pain. When compared to fentanyl, sufentanil is superior in blocking stress responses and may be suited for longer surgical procedures, although it is yet a shorter-acting compound.

Due to the high potency of the three compounds of interest, it was decided that the targeted cleaning assay limits had to be reduced to the lowest possible level, taking into consideration the sensitivity of the developed method. A literature review revealed that a few existing HPLC methods applied mostly to plasma samples with inadequate sensitivity for the purpose of this study and no validation of cleaning methods could be found. This work is based on a previously developed method [9] for the assay of fentanyl and related substances, since all three active compounds are structurally very similar.

2. Experimental

2.1. Materials

HPLC grade acetonitrile from Mallinckrodt (Phillipsburg, NJ) was used to prepare the mobile phase. Perchloric acid of reagent grade quality from Mallinckrodt and in-house Milli-Q water were used to prepare the aqueous component of the mobile phase. Sodium 1-decane sulfonate was obtained from Sigma (St. Louis, MO). Fentanyl citrate raw material was obtained from Mallinckrodt. Alfenta injection (500 μg/ml) and SUFENTA injection (50 μg/ml) were obtained from Janssen (Titusville, NJ). Super POLX 1200 wipers (10.2 × 10.2 cm pads), latex powder-free gloves, polypropylene disposable centrifuge tubes, and 0.45 μm Nalgene PTFE filters were obtained from VWR Scientific Products (West Chester, PA). The structures and names of these active pharmaceutical ingredients (APIs) are given in Fig. 1.

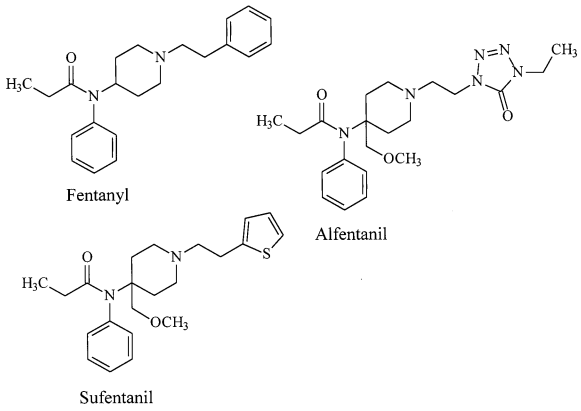

Fig. 1. Structures of fentanyl, alfentanil, and sufentanil.

2.2. Equipment

An HPLC system consisting of a Hitachi model L6200a intelligent pump plus an Alcott model 728 autosampler, ACCESS*CHROM 1.9 chromatography data software with a PE Nelson A/D interface system, and an Applied Biosystems 759A variable wavelength UV detector were used. All separations were achieved using 15 cm × 4.6 mm ID, 5μ C18 Inertsil columns (obtained from Phenomenex or Alltech). The mobile phase, which consisted of different mixtures of perchloric acid (0. 23% w/v) containing sodium 1-decane sulfonate (10 mM) and acetonitrile, was degassed with helium prior to use. All sample and standard solutions were chromatographed at ambient temperature with varying mobile phase compositions, detection wavelengths, and flow rates depending on the analyte. Peak height responses were used for the quantitation of sufentanil (as opposed to peak area responses for the other two components) due to the better reproducibility at the low quantitation levels. The required chromatographic conditions for fentanyl, alfentanil, and sufentanil are listed in Table 1.

2.3. Preparation of the mobile phase

Perchloric acid (70%, w/w, 2.0 ml) was carefully added to 1.0 l water and mixed well. Approximately 2.5 g sodium 1-decane sulfonate was dissolved in the resulting solution, which was then mixed to the desired composition based on the assay performed (see Table 1). The mobile phase was appropriately degased before used.

Table 1
Summary of chromatographic conditions

Conditions	Compound of interest		
	Fentanyl	Alfentanil	Sufentanil
Mobile phase composition (perchloric[a]:acetonitrile, v/v)	60:40	60:40	55:45
Ultraviolet detection (nm)	205	208	230
Flow rate (ml/min)	1.0	1.5	1.5
Quantitation peak response	Area	Area	Height
Assay quantitation levels (ng/ml)	10	50	15
Assay detection levels (ng/ml)	2	10	10
Assay limits (ng/swab)	20	100	100

[a] Perchloric: aqueous perchloric acid 0. 23% (w/v) containing 1-decane sulfonate (10 mM).

2.4. Preparation of standard solutions

2.4.1. Fentanyl

A stock solution at 400 μg/ml was prepared by dissolving approximately 40 mg fentanyl citrate in 100 ml water. An aliquot (1.0 ml) of the stock solution was diluted twice in 100 ml water to provide a concentration of about 0.04 μg/ml. A final dilution of 25 ml of the resulting solution in 100 ml water provided the standard solution of 10 ng/ml fentanyl citrate.

2.4.2. Alfentanil

A portion (1.0 ml) of Alfenta injection (alfentanil hydrochloride, 500 μg/ml of the free base) was diluted in 50 ml water. An aliquot (1.0 ml) of the resulting solution was diluted in 200 ml water to provide a concentration of about 50 ng/ml alfentanil (free base).

2.4.3. Sufentanil

A portion (1.0 ml) of Sufenta injection (sufentanil citrate, 50 μg/ml of the free base) was diluted in 100 ml water. An aliquot (3.0 ml) of the resulting solution was diluted in 100 ml water to provide a concentration of about 15 ng/ml sufentanil (free base).

2.5. Preparation of swab samples

Super POLX 1200 wipers were cut into 5×10 cm^2 pads and folded in half (5×5 cm^2) before used. Then each swab was slowly wet with water and let sit for about 10 s to absorb the solvent. Any excess solvent was removed by squeezing the swab. As the test surface, circular 316 stainless steel plates (with an area of 25.8 cm^2 each) polished to a near mirror finish on one face were utilized. A 10-cm long handle was attached to each plate. The actives were spiked onto the stainless steel plates that were then swabbed by spiraling the wet swab from the outer area of the circle to the center. The swabbing procedure was performed twice clockwise, then twice counterclockwise. The swab was folded again in half and the procedure was repeated, finishing by folding the swab again and dabbing the center of the circular area. Finally, the swab sample was placed in a centrifuge tube.

2.6. Extraction of swabs

All swabs were placed in 50 ml centrifuge tubes. Extraction solvent (water, 10 ml) was added to the swab sample, which was then mechanically shaken for 15–20 min, rotating the tube after about half its shaking time. Then it was hand-shaken vigorously in a horizontal fashion about ten times and the extract filtered through a 0.45 μm PTFE filter, discarding the first 1–2 ml of the filtrate.

2.7. Limit test solutions

Limit of detection (LOD) test solutions in water were prepared at concentrations of approximately

2 ng/ml for fentanyl citrate, and 10 ng/ml (free base concentration) for both alfentanil hydrochloride and sufentanil citrate by dilutions of the corresponding standard solutions.

Table 2
Limits of detection and quantitation for fentanyl, alfentanil and sufentanil

Active ingredient	LOD		LOQ	
	μg/ml	μg/swab	μg/ml	μg/swab
Fentanyl	0.002	0.02	0.01	0.10
Alfentanil	0.01	0.10	0.02	0.20
Sufentanil	0.003	0.03	0.015	0.15

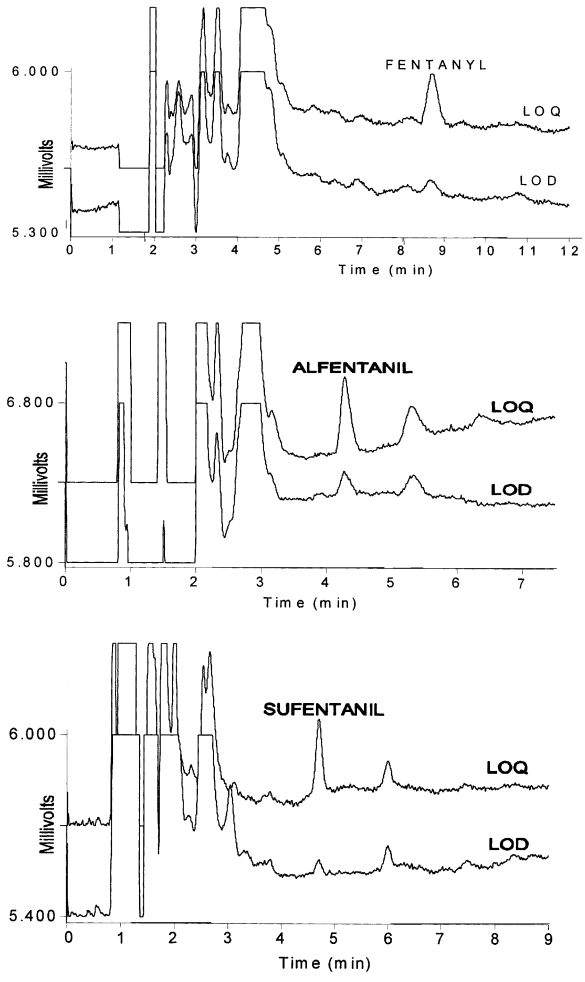

Fig. 2. Example chromatograms at the LOD and LOQ levels.

3. Results

3.1. Development of the chromatographic separation

As mentioned previously, a starting point for the development of the cleaning assay for all three drugs was our previous work on the assay method for Fentanyl [9]. Efficient chromatography and high sensitivity was achieved by using aqueous perchloric acid and acetonitrile (ACN) as the mobile phase, with varying detection wavelengths, based on the response of the active, and an injection volume of 200 μl. The amount of organic modifier was adjusted so that the cleaning assay run time could be reduced for faster analysis of the cleaning samples. However, based on preliminary experiments performed in our laboratory, it was shown that aged extractables from the swab material degraded over time to compounds that interfered with the quantitation of the active compounds. Therefore, a second ion-pairing agent (sodium 1-decane sulfonate) was added to the mobile phase in order to achieve better separation from the interfering compounds and the flow rate was varied to optimize the active retention on the column. Water was chosen as the swabbing and extraction solvent because of the high solubility of all three compounds in this solvent and the low content of water-soluble material present in the wipers, and 200 μl of the test solution was injected in order to achieve sufficient sensitivity.

3.2. Limits of detection (LOD)/quantitation (LOQ)

The detection and quantitation limits for the assays of fentanyl citrate, alfentanil, and sufentanil are shown in Table 2. All calculations for these limits were based on signal-to-noise ratios (approximately equal to 3 for LOD and 10 for LOQ). An example chromatogram for both levels and all three actives is shown in Fig. 2.

Table 3
Parameters of linearity of fentanyl, alfentanil, and sufentanil

Compound	Calibration range		y-Intercept	Slope	r^2	Response factor
	Nominal analytical conc. (%)	Conc. (ng/ml)				
Fentanyl citrate	100–320	10–32	−649.537	461.826	0.99223	423.32
Alfentanil	40–400	20–200	−91.4748	257.478	0.99813	256.13
Sufentanil	100–320	15–48	5.02399	17.4112	0.99014	17.544

3.3. Range of linearity

The linearity parameters of the curve for the fentanyl and alfentanil peak area responses and the sufentanil peak height responses versus their concentrations were studied in the concentration range corresponding to about 100% to over 300% of the quantitation limits of 10, 20, and 15 ng/ml, respectively (Table 3).

3.4. Selectivity

Fig. 3 shows chromatographic overlays of injections of sample extracts corresponding to the assay limits of detection and quantitation, diluent (water), and a blank swab extract for all three active components. No interference was observed with the analysis of cleaning samples.

3.5. Accuracy/recovery/intermediate precision

To measure accuracy and recovery, each component was spiked onto the stainless steel plates by pipetting 0.5 ml of stock solutions with the appropriate concentrations. The solvent used to make the spiking solutions was methanol:water (50:50, v/v). The solutions were dispersed dropwise so that small beads of solvent covered the surface of the plate. Solutions were air-dried (approximate time was 2 h). Three spiked plates were prepared at each level of quantitation and detection for all three actives. Due to the higher concentration of alfentanil in the drug product, compared to fentanyl and sufentanil, an additional cleaning level was studied. Analyst-dependent (intermediate) precision was determined by a repeat assay of accuracy/recovery experiments by a second analyst on a different HPLC system. At the levels of detection, all actives were detected in all preparations by both analysts. The recovery results at the assay levels of quantitation are shown in Table 4.

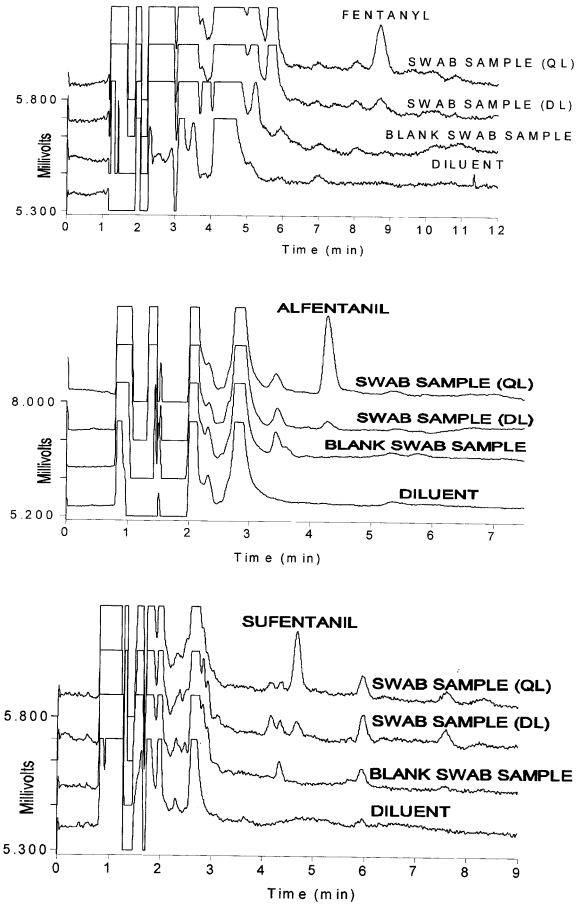

Fig. 3. Chromatographic overlay of samples at the quantitation and detection levels, diluent, and blank swab samples.

Table 4
Accuracy/recovery/intermediate precision studies for fentanyl citrate, alfentanil and sufentanil

	Recovery (%)			
Fentanyl level	10 ng/ml (0.1 μg/swab)			
	Analyst # 1	Analyst # 2		
Mean (3)	97.3	93.5		
%R.S.D.	3.4	6.4		
Mean (6)	95.4			
%R.S.D.	5.1			
Alfentanil level	50 ng/ml (0.5 μg/swab)		100 ng/ml (1.0 μg/swab)	
	Analyst # 1	Analyst # 2	Analyst # 1	Analyst # 2
Mean (3)	76.4	87.6	75.1	91.2
%R.S.D.	5.4	6.6	7.4	6.8
Mean (6)	82.0		83.2	
%R.S.D.	9.3		12.4	
Sufentanil level	15 ng/ml (0. 15 μg/swab)			
	Analyst # 1	Analyst # 2	Analyst # 3	
Mean (3)	81.0	99.2	78.8	
%R.S.D.	4.4	5.4	7.4	
Mean (9)		86.3		
%R.S.D.		12.3		

Table 5
System precision for fentanyl citrate, alfentanil, and sufentanil working standard and sample solutions

	Injection Fentanyl		Alfentanil		Sufentanil	
Standard	Sample	Standard	Sample	Standard	Sample	
Mean (6)	4496	4155	13043	12374	263	276
%R.S.D.	5.0	6.3	2.4	1.8	3.5	2.6

3.6. System precision

The study was performed by making six replicate injections of each working standard solution and of a recovery sample solution prepared at the assay level of quantitation. The results are summarized in Table 5.

3.7. Stability of analytical solutions

The stability of standard solutions was monitored by analyzing aged solutions maintained at room temperature and protected from light, against freshly prepared standards. The results demonstrated that actives in working standard solutions (assay concentrations) were stable for at least 4 days (Table 6).

Stability of swab samples was determined by spiking swabs directly with solutions of the appropriate active and assaying immediately after its preparation and again after aging for several days. Following the procedures outlined previously, the swabs were extracted in duplicate immediately after preparation and after aging for several days. As shown in Tables 7 and 8, swab samples and extracts (prepared at the quantitation

level) were found to be stable for at least 4 and 3 days, respectively, without any refrigerated conditions required. In addition, swab samples and swab extracts, spiked at the detection level and stored under the same conditions yielded detectable fentanyl responses. Therefore, it was concluded that both swab extracts and swab samples were stable for at least 3 and 4 days, respectively, when stored at room temperature. During the stability studies no additional peaks developed and no changes in the chromatography were observed.

4. Conclusions

The developed swab method for cleaning control during fentanyl, alfentanil and sufentanil drug product manufacturing was validated and shown to be selective and linear from 100% to at least

Table 6
Stability of working standard solutions

Active	Potency (%)				
	Initial	2 Days	3 Days	4 Days	6 Days
Fentanyl	100.0	107.3	–	–	100.1
Alfentanil	100.0	106.5	–	103.3	–
Sufentanil	100.0	–	95.7	–	93.5

Table 7
Stability of swab samples

Active	Sample recovery (%)				
	0 Days	2 Days	3 Days	4 Days	6 Days
Alfentanil (50 ng/ml level)	97.3	95.4	–	100.1	–
Alfentanil (100 ng/ml level)	95.0	92.0	–	97.2	–
Sufentanil	106.9	–	107.9	–	110.2
Fentanyl	Room temperature		Refrigerated (5°C)		
	4 Days	7 Days	4 Days	7 Days	
	95.5	93.8	105.5	105.7	

Table 8
Stability of swab extracts

Active	Sample extract recovery (%)				
	0 Days	2 Days	3 Days	4 Days	6 Days
Alfentanil (50 ng/ml level)	78.5	80.6	–	84.2	–
Alfentanil (100 ng/ml level)	77.6	77.0	–	79.4	–
Sufentanil	81.4	–	77.2	–	–
Fentanyl	97.0	106.4	–	–	108.5

300% of the concentration of the actives at their corresponding quantitation limits, with a coefficient of determination (r^2) greater than 0.99. The detection levels per swab area were set at 0.2% or lower, when compared to the concentration of the active substances in the respective drug products and quantitative recoveries for all three components at the quantitation levels were obtained. Repeat recovery studies on a different system by a second analyst demonstrated the precision of the methods and more than 80% of the corresponding active substance was recovered from the stainless steel surface (average of two analysts). No interference was observed in injections of diluent or blank extracts and all swab samples/extracts and standard solutions were shown to be stable for at least 2 days when stored under ambient laboratory conditions.

Acknowledgements

The authors wish to acknowledge Dr Tanya Toney-Parker and the rest of the staff at the AA1 Formulations Development Laboratory (FDL) for their support.

References

[1] The United States Pharmacopeia, 23rd revision, United States Pharmacopeial Convention, Incorporated, Rockville, MD, 1995, pp. 654-655.
[2] I. Krämer, S. Balzulat, H. Lammers, Krankenhauspharmazie 12 (6) (1991) 231–233.
[3] T.D. Wilson, T. Maloney, W.B. Amsden, J. Chromatogr. 445 (1988) 299–304.
[4] H. Theuer, G. Scherbel, U. Windsheimer, Krankenhauspharmazie 12 (6) (1991) 233–245.
[5] K. Kumar, D.J. Morgan, D.P. Crankshaw, J. Chromatogr. 419 (1987) 464–468.
[6] Clinical Pharmacy, 10 (1991) 635-637.
[7] Clinical Pharmacy 6 (1987) 275–282.
[8] P.J. Roos, J.H. Glerum, L.W. Meilink, Pharm. Weekbl. Sci. 14 (4) (1992) 196–200.
[9] J. Lambropoulos, G.A. Spanos, N.V. Lazaridis, T.S. Ingallinera, V.K. Rodriguez, J. Pharm. Biomed. Anal. 20 (1999) 705–716.