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Sensitive and selective assay for fentanyl using gas chromatography with mass selective detection

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Abstract

A modified gas chromatographic assay, using mass-selective detection, has been developed for the quantitation of fentanyl in swine serum. Fentanyl and sufentanil, the internal standard, were extracted using a single-step liquid–liquid extraction with dichloromethane. Sensitivity and selectivity were improved by using electron-impact ionization (EI) in the selected-ion monitoring (SIM) mode, where fentanyl and sufentanil were monitored using the fragment ions at m/z 245 and 289, respectively. The limit of quantitation (LOQ) is 0.05 ng/ml, using 1 ml of sample, with a C.V. of 10.8% and a signal-to-noise ratio of 29. Standard curves were linear ($r^2 = 0.999$) over the working range of 0.05–1.5 ng/ml, using $1/y^2$ as a weighting factor. Recoveries averaged 69.8 ± 4.7%, 91.0 ± 13.0% and 90.9 ± 10.3% at serum concentrations of 1.5, 0.5 and 0.1 ng/ml, respectively. Intra- and inter-day variances, were <12% at 0.1 ng/ml, and <10% at concentrations of 0.5, 1 and 1.5 ng/ml. Bias was 6.2% at the LOQ and ≤12.8% at every other standard curve concentration. Applicability of the assay is demonstrated for the pharmacokinetic study of transdermally administered fentanyl in a postoperative swine.

Keywords: Fentanyl

1. Introduction

Fentanyl is a synthetic narcotic analgesic used in anaesthesia both as a preanaesthetic, and postoperatively to control pain. It exerts its effect primarily on the μ-opioid receptor and is ca. 80 times more potent than morphine [1]. Fentanyl has a short duration of action, minimal cardiovascular effects and a high therapeutic index [2]. For effective postoperative analgesic therapy, the use of fentanyl transdermal delivery systems has come into common practice. This method of drug administration results in very low blood concentrations (i.e., ≤1 ng/ml); therefore, selective and sensitive analytical methods capable of detection in the low picogram range are required for the quantitation of drug in biological matrices.

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Several methodologies are available for the determination of fentanyl. These techniques include radioimmunoassay (RIA) [3–5], and gas chromatography (GC) using ultraviolet [6], thermionic [7], electron capture [8], nitrogen–phosphorus [2,9–11] and mass spectrometric [2,12–15] detection. High-performance liquid chromatographic (HPLC) methods are also available [16,17]. The RIA methods are sensitive (e.g., limit of detection (LOD): 0.025 ng/ml [4]), but they can be highly variable and subject to marked over-estimation [3]. They can also provide significantly different results when compared with GC techniques [18]. RIA has also been reported to lack selectivity [6], with cross-reactivity to various biological components [12] and fentanyl metabolites [7]. The administration of tritium-labeled fentanyl to patients, for RIA assay, has also been reported to be disadvantageous [10]. GC methods have good sensitivity (e.g., LOD: 0.08 ng/ml [10] or 0.05 ng/ml [2]) but they require either derivative formation [8], rigorous glassware deactivation [2,11] or intensive sample work-up including back-extraction [2,7,15]. A sensitive HPLC method has been reported with an LOD of 0.158 ng/ml in blood [17]. Rapid but insensitive HPLC [16] and refractometric [19] methods are also available to screen for possible tampering with pharmaceutical preparations of fentanyl.

In the present study, a selective and sensitive assay method (LOQ: 0.05 ng/ml) is presented for the quantitation of fentanyl in the serum samples of swine. The procedure does not require prior glassware deactivation and employs a single-step liquid–liquid extraction followed by GC with mass-selective detection.

2. Experimental

2.1. Materials

Fentanyl citrate and sufentanil citrate were provided by the Bureau of Drug Research, Health and Welfare Canada, Health Protection Branch (Ottawa, Canada). The fentanyl patches (Duragesic 50 fentanyl transdermal system, Janssen Pharmaceutica, Mississauga, Canada) were obtained from University Pharmacy (University of British Columbia, Vancouver, Canada). Ketamine HCl (Ketalean HCl inj.) was purchased from MTC (Cambridge, Canada), and isoflurane (Forane) from Anaquest (Mississauga, Canada). Chlorhexidine gluconate 2% surgical soap (Hibitine) was purchased from Ayerst Laboratories, Division of Ayerst, McKenna and Harrison Inc. (Montreal, Canada). The endotracheal tube used for intubation was a Magill-type cuffed tracheal tube with a 7 mm I.D. (Sheridan, Argyle, NY, USA). Sodium hydroxide (ACS-certified) was purchased from Fisher Scientific (Nepean, Canada); toluene, and dichloromethane (distilled in glass) from Caledon Laboratories (Georgetown, Canada); and triethylamine (TEA) (Sequanal grade) from Pierce (Rockford, IL, USA). HLPC-grade water was used throughout our analytical procedures and was prepared in our laboratory using a Milli-Q Water System (Millipore, Bedford, MA, USA).

2.2. Swine experiments

All animal experiments were conducted in accordance with the standards defined in the Canadian Council of Animal Care's Guide to the Care and Use of Experimental Animals, Volume 1 and under approved institutional animal care protocols. Yorkshire cross white female pigs (weighing 26.2 ± 2.1 kg, mean $\pm$ S.D., $n = 4$) were used in the experiments. They were singly housed in 2×2 m pens on an elevated Tenderfoot flooring with ad libitum access to water and hog ration fed to a maximum of 2 kg per day. Animals were behaviorally assessed by blinded analysis of videotapes recorded at various time intervals before and after surgery and in the presence and absence of surgical pain and narcotic analgesia. The animals shared fenceline contact with another familiar pig. A transdermal fentanyl patch (Duragesic 50 μg/h) was applied to the intrascapular area after skin cleansing with chlorhexidine gluconate 2% surgical soap and 95% isopropyl alcohol. An occlusive dressing was placed over the patch to mask the area from observation during videotapes analysis. Four

days prior to surgery the first fentanyl patch was applied for a 24 h period as a control experiment to assess behaviour with narcotic in the absence of surgical pain. On the day of surgery, following a 12–14 h fast, pigs were sedated with ketamine 20 mg/kg by intramuscular injection and anaesthesia induced with 4% isoflurane in oxygen by mask. Following intubation with a cuffed 10 mm endotracheal tube, anaesthesia was maintained at 1–2% isoflurane in 45% oxygen and air. Following anaesthetic induction, a second fentanyl patch was applied, a left hemithorax aseptically prepared and a left lung allograft transplant performed by an experienced transplant surgeon. Recovery from surgery lasted 4 to 5 h after the induction of anaesthesia. Blood samples, for serum fentanyl analysis, were collected aseptically via a vascular access catheter port implanted in the anterior vena cava, one day and four days after the application of the first patch (control) and 0, 4, 8, 24, 48 and 72 h after the application of the second patch.

2.3. Stock solutions and sample preparation

Aqueous stock solutions of fentanyl citrate (50 ng/ml and 5 ng/ml) and of sufentanil citrate (10 ng/ml) were prepared using HLPC-grade water. Weights were normalized to free base and the stock solutions were kept protected from light, at 4°C, until use.

The extraction procedures were carried out with duplicate samples. Serum samples (0.05–1 ml) were pipetted into clean 15 ml borosilicate test tubes and 100 μl (1 ng) of sufentanil internal standard solution (10 ng/ml) added. The pH of the biological matrix was adjusted to ca. 13 with 0.5 ml of 1 *M* NaOH, and the final volume adjusted to 2.0 ml by adding appropriate volumes of HPLC-grade water. An aliquot of 6 ml of dichloromethane containing 0.5 *M* TEA was added and the test tubes closed with screw caps lined with polytetrafluoroethylene (PTFE). The tubes were mixed on a Labquake rotary shaker (Labindustries, Berkeley, CA, USA) for 20 min, and then kept at −20°C for 15 min to break any emulsion formed during mixing. The samples were subsequently centrifuged at 3000 *g* for 10 min using an IEC HN-SII centrifuge (Damon/IEC Division, Needham Heights, MA, USA) and the upper aqueous phase aspirated and discarded. The organic phase was transferred to another set of clean test tubes and evaporated to dryness under a gentle stream of nitrogen, in a 30°C water bath (Haake D1 Type 001-3950 heater/regulator; Haake, Berlin, Germany). The residues were reconstituted with 50 μl of toluene containing 12.5 m*M* TEA and mixed thoroughly using a Maxi-Mix II Model M37600 vortex mixer (Thermolyne/Sybron Corporation, Dubuque, IA, USA). The reconstituted samples were transferred into 0.15 ml borosilicate glass autosampler vial inserts and 2 μl was injected into the GC.

2.4. Gas chromatography–mass-selective detection

Fentanyl was analysed using a Hewlett-Packard 5890 Series II GC equipped with a Hewlett-Packard Model 7673 automatic sampler (Hewlett-Packard, Avondale, PA, USA). Samples (2 μl) were injected in the splitless mode through a Thermogreen LB-2 11 mm septum (Supelco, Bellefonte, PA, USA) into a split/splitless capillary inlet system. In order to minimize drug binding to the active sites of the glass insert, a Hewlett-Packard single-tapered (HP Part No.: 5181-3316) deactivated borosilicate glass injection port liner (80 × 4 mm I.D.) was used with a gold-plated inlet seal at the bottom of the inlet system. To improve sample volatilization, a 2–3 mm silanized glass wool plug was placed in the middle of the liner. Chromatographic separation of fentanyl from sufentanil was achieved using an HP Ultra-2 cross-linked 5% phenylmethyl silicone fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness) (Hewlett-Packard, Palo Alto, CA, USA). GC conditions were as follows: injection port temperature 280°C; initial oven temperature 100°C for 1 min, ramped to 280°C at a rate of 70°C/min and held for 10 min, resulting in a total run time of 13.57 min. Helium (Ultra High Purity, Matheson Gas Products, Edmonton, Canada) was used as the carrier gas with a total inlet flow of 30 ml/min and septum purge of ca. 0.8 ml/min. A 1 ml/min

column flow, measured at the initial oven temperature, was provided by a 70 kPa column head pressure. A Hewlett-Packard 5971A MSD was used for analyte detection with a transfer line temperature of 290°C. Using the electron-impact (EI) ionization mode, the emission current was 300 μA, and the ionization energy was a factory set value of 70 eV. To increase sensitivity, the MSD was manually tuned to the molecular fragments of the mass-scale calibrant perfluorotributylamine (FC-43) of m/z 131, 219 and 264. Full-scale mass scanning (SCAN) was performed for qualitative purposes to determine the fragmentation pattern of fentanyl and sufentanil. The mass spectra of fentanyl and sufentanil showed the base peaks at m/z 245 and m/z 289, respectively. Fentanyl was quantitated by selected-ion monitoring (SIM) of a fragment at m/z 245 (group 1), whereas sufentanil (internal standard) was monitored via its fragment ion at m/z 289 (group 2). The dwell time was adjusted to 600 ms in both groups, with mass spectrometric high resolution, providing 1.58 scan cycles/s for the compounds. While the selectivity of the detection of fentanyl was improved by collecting the ion current due to the fragment ion at m/z 245, sensitivity was enhanced by programming the electron multiplier (EM) voltage of the MSD during the elution time of fentanyl and sufentanil. The default EM voltage value (ca. 1700 V) acquired by tuning the MSD was ramped by +1200 V at 10.4 min and held for 1.8 min and then reduced back to the original tune value. In this way, 2 pg of fentanyl (LOQ) could easily be detected without a significant increase in baseline noise.

2.5. Calibration curve

Aliquots (0.5 ml) of blank swine serum were pipetted into clean sets of 15 ml borosilicate test tubes. A six-point calibration curve was prepared by spiking the serum samples with appropriate volumes of the 50 ng/ml and the 5 ng/ml fentanyl aqueous stock solutions yielding concentrations of 0.05, 0.1, 0.2, 0.5, 1, and 1.5 ng/ml. A-100 μl (1 ng) volume of the 10 ng/ml sufentanil (internal standard) aqueous stock solution was added to each tube. The calibration curve was plotted as fentanyl concentration vs. the ratio of the peak area count of fentanyl to that of sufentanil. Quantitation of fentanyl was achieved by using a weighted linear regression analysis with a weighting factor of $1/y^2$.

2.6. Recovery

Recovery of fentanyl from swine serum was studied at three calibration levels. Two sets of 15 ml clean borosilicate test tubes were prepared as the *control* and *recovery* groups. Into the test tubes of the *control* group, 0.5 ml blank serum was pipetted and spiked with 1.5 ng, 0.5 ng, or 0.1 ng of fentanyl using appropriate volumes of the fentanyl aqueous stock solutions. The internal standard, sufentanil (1 ng), was also added to the tubes and they underwent the previously described extraction procedure. Into the test tubes of the *recovery* group, the same volumes of the fentanyl aqueous stock solutions were added and evaporated to dryness under a gentle stream of nitrogen in a water bath, at 30°C. Into another set of clean test tubes, 0.5 ml blank serum was pipetted and spiked with 1 ng sufentanil. These test tubes also underwent the extraction procedure detailed above, following which the organic phase was quantitatively transferred into the test tubes containing the dried residues of the fentanyl in the *recovery* group. From this point, the *control* and the *recovery* groups were processed together according to the assay procedure described in this paper.

2.7. Method validation

Intra-day and inter-day variance

Intra-day and inter-day variances were both determined at four calibration levels using the same serum stock solutions, which were prepared as follows. Two sets of 5.0 ml serum stock solutions were prepared by spiking blank serum samples with appropriate volumes of the fentanyl aqueous stock solution to yield the final concentrations of 50 ng/ml and 5 ng/ml, respectively. These two serum stock samples were used during the method validation procedure.

Intra-day variance: on the same day, on five separate occasions, aliquots of the 50 ng/ml and the 5 ng/ml serum stock solutions, representing 1.5 ng/ml, 1 ng/ml, 0.5 ng/ml, and 0.1 ng/ml calibration levels, were analysed with the extraction procedure detailed above. The variance was assessed by comparing the results of the five measurements and determining the coefficient of variation (C.V.).

Inter-day variance: on five successive days, one occasion every day, aliquots of the 50 ng/ml and the 5 ng/ml serum stock samples, representing 1.5 ng/ml, 1 ng/ml, 0.5 ng/ml and 0.1 ng/ml calibration levels, were analysed with the extraction procedure detailed above. The variance was assessed by comparing the results of the five measurements and determining the C.V.

Accuracy

Five calibration curves were prepared and analysed with the above described extraction procedure. Weighted linear regression analyses were carried out (weighting factor: $1/y^2$) and the six calibration concentrations were back-extrapolated using the regression parameters obtained. Accuracy was determined by expressing the percentage difference (bias) between the average measured concentrations and the average added concentrations at the six calibration levels.

3. Results and discussion

3.1. Sample preparation and GC–MS

The analytical assay procedure presented in this paper is based upon a previously reported GC–MS assay method [13] with modifications. The internal standard flurazepam was replaced with sufentanil, a structural analogue of fentanyl. Sufentanil also proved to be a much better compound for mass-selective detection, because it provided a selective and well-defined fragment at m/z 289, whereas the prominent ion of flurazepam was in the non-specific low-mass range (viz. m/z 86). The extraction solvent, *n*-butyl chloride–5% isopropyl alcohol, was replaced with dichloromethane, resulting in good analyte extractability and rapid sample drying. A single-step extraction could be employed because interferences with any co-extracting endogenous components were eliminated by selectively monitoring ion fragments of fentanyl and sufentanil. Sensitivity of the assay was increased by using a low sample-reconstituting volume (50 μl) as well as by enhancing the chromatographic response of fentanyl (ca. 1.5–2 fold) by placing a silanized glass wool plug in the injection port liner.

Fentanyl has been reported to bind to the active sites of glassware [11], which could result in considerable loss of drug during analysis. However, information in this regard is conflicting because another study, using glass and plastic containers during the fentanyl analysis, reported no significant loss of the drug after 48 h [20]. To reduce potential loss of the drug due to adsorption to glass, we used borosilicate glassware during our assay procedure. To further lessen possible adsorption losses, 0.5 *M* TEA was added to the dichloromethane extraction solvent, and 12.5 m*M* TEA to the toluene reconstituting solvent. In the recovery experiments, the alkalinity of the 12.5 m*M* TEA added to the reconstituting solvent also served to convert the dried fentanyl citrate salt residues to the free base.

A representative total-ion chromatogram (SCAN mode) of 200 ng fentanyl and 100 ng sufentanil spiked in 0.5 ml blank swine serum is presented in Fig. 1. The retention times of fentanyl and sufentanil are 11.05 and 12.12 min, respectively. The peaks are well-separated and the superimposed total-ion chromatogram of blank serum shows no interference at the retention times of the peaks. The mass spectra of fentanyl and sufentanil are presented in Fig. 2. Fentanyl underwent extensive fragmentation, showing the molecular ion at m/z 336 and the base peak fragment ion at m/z 245. Additional ion fragments are m/z 146, 189, 105, which are in accordance with the literature data [21,22] (Fig. 2A). Fentanyl was quantitated by monitoring the single-ion current of m/z 245, which provided very specific signal detection. Using this ion, the signal-to-noise ratio was 29 at the LOQ (i.e., 0.05 ng/ml) (Fig. 3). Sufentanil also underwent exten-

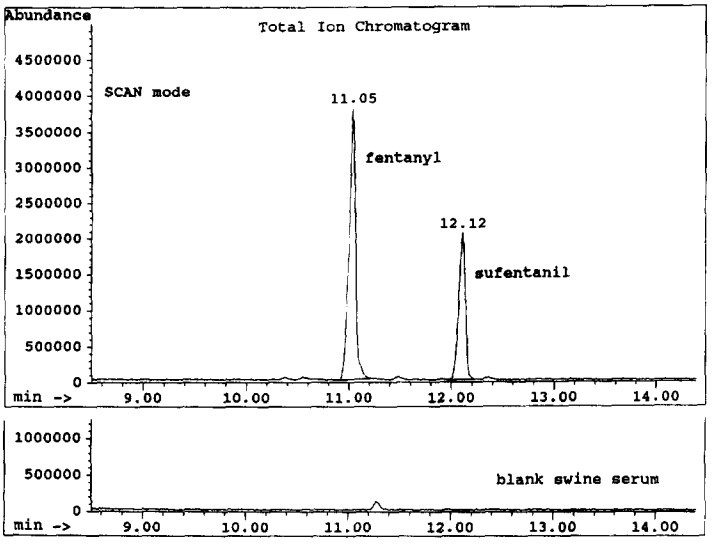

Fig. 1. Superimposed total-ion chromatograms (SCAN mode) of blank swine serum and 0.5 ml of blank serum spiked with 200 ng fentanyl and 100 ng sufentanil (internal standard).

sive fragmentation, showing the base peak fragment ion of m/z 289 with no detection of the molecular ion of m/z 386. Additional fragments are m/z 140 and 106 (Fig. 2B). Sufentanil was selectively monitored by collecting the single ion current of m/z 289.

A representative SIM chromatogram of 0.05 ng/ml fentanyl (LOQ) and 1 ng/ml sufentanil is shown in Fig. 3. No interfering compounds were found at the retention times of fentanyl and sufentanil. The step-like shift in the baseline occurring at 10.57 min is the result of increasing the EM voltage by 1200 V. A similar shift can be seen at 11.4 min due to group-switching in the MSD from monitoring m/z 245 (group 1) to m/z 289 (group 2). In this instance, there is a drop in baseline as a result of a lower level of background noise.

3.2. Calibration curve

The six-level calibration curve of fentanyl in swine serum showed good linearity over the working concentration range of 0.05–1.5 ng/ml. Using a weighting factor of $1/y^2$ during the linear regression analyses, the calibration curve was best described by the following equation: $y = 1.12x - 0.03$, $r^2 = 0.999$. If no weighting factor or $1/y$ weighting was used, the results obtained at the lower range of the calibration curve (i.e., ≤ 1 ng/ml) had the tendency to be over-estimated, whereas the other calibration levels had good accuracy. When a $1/y^2$ weighting factor was used, the accuracy of the higher calibration levels (i.e., ≥ 1 ng/ml) was compromised slightly in favour of the lower levels, but good linearity coupled with good coefficient of determination (r^2) were maintained.

3.3. Recovery

The extraction recovery of fentanyl from serum was determined by comparing the quantitation results of the three fentanyl calibration levels (i.e., 1.5 ng/ml, 0.5 ng/ml, 0.1 ng/ml) obtained from the *control* and *recovery* groups. The *control* group underwent the extraction procedure whereas the *recovery* group did not. The extraction recovery was expressed as the percentage of the fentanyl concentration of the *control* group to that of the *recovery* group. Because the free base form of fentanyl was not available to us, the aqueous solutions of the citrate salt of the compound were used for the

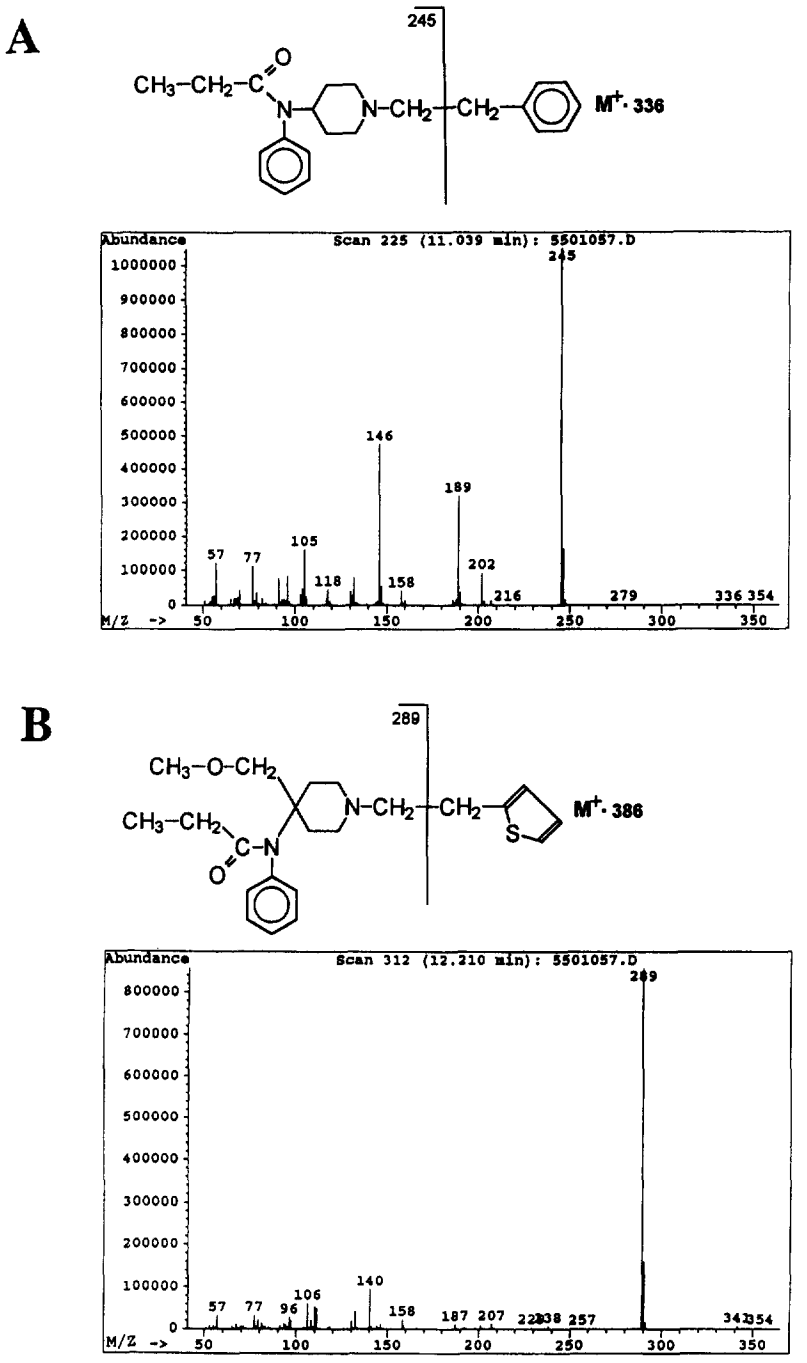

Fig. 2. EI mass spectra of fentanyl (A) and sufentanil (B), indicating the molecular ion ($M^{+}\cdot$) for fentanyl (A) and the fragment ions used for quantitation.

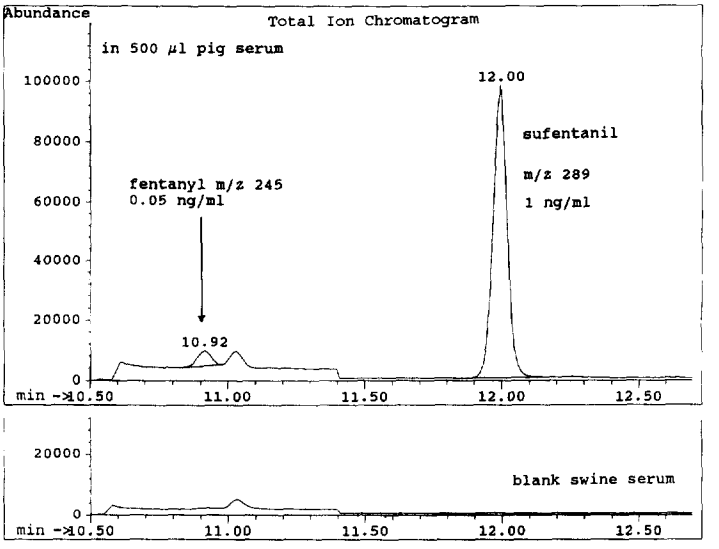

Fig. 3. Superimposed single-ion chromatograms (SIM mode) of blank swine serum and 0.5 ml of blank serum spiked with 0.05 ng/ml fentanyl (LOQ) and 1 ng/ml sufentanil (internal standard).

recovery studies. After the appropriate aliquots of the fentanyl aqueous stock solutions were evaporated, the residual citrate salt was converted to the free base by adding 12.5 m*M* TEA to the toluene reconstituting solvent. Another reason for adding TEA to the toluene was to reduce possible fentanyl binding to active sites on glass surfaces. The recovery values of fentanyl from swine serum, at the calibration levels examined, were as follows (mean ± S.D.): 1.5 ng/ml: 69.8 ± 4.7% ($n = 6$), 0.5 ng/ml: 91.0 ± 13.0% ($n = 6$), 0.1 ng/ml: 90.9 ± 10.3% ($n = 4$). The 1.5 ng/ml calibration concentration showed a decreased recovery value, suggesting concentration-dependent extraction efficiency. To examine for possible reduced recovery, the serum samples of the 1.5 ng/ml concentration in the *control* group were extracted a second time with an additional 3.0 ml dichloromethane. No fentanyl could be detected in this second serum extract, indicating complete extraction of the drug with the first 6.0 ml aliquot of dichloromethane.

3.4. Intra-day, inter-day variance and accuracy

The intra-day and the inter-day variance of the assay are presented in Table 1.

Inter-day variance was determined in the following way: 1.5, 1, 0.5, and 0.1 ng/ml calibration concentrations were extracted five times on the same day, and the C.V. between the results calculated. Intra-day variance did not exceed 10.7%.

Inter-day variance was determined in the following way: 1.5, 1, 0.5, and 0.1 ng/ml calibration concentrations were extracted on five successive days, one experiment every day. The C.V. between the results were determined, and they did not exceed 11.5%.

The accuracy of the assay was determined by analysing five calibration curves (Table 2). Each calibration concentration was back-extrapolated using the regression parameters obtained (i.e., slope, intercept) and these values (measured amount) were compared to the actual amounts of drug added. Accuracy (bias, %) was expressed as: [(measured amount/added amount) × 100] − 100, with (+)bias representing over-estimation, (−)bias representing under-estimation. The use of the weighting factor of $1/y^2$ resulted in a good overall accuracy throughout the calibration curve. When the variance of the calibration points were analysed using multiple samples at each concentration ($n = 5$), the C.V. did not

Table 1
Intra-day and inter-day variance of the assay in swine serum (n = 10 at each concentration)

Spiked concentration (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)	
	Intra-day variance	Inter-day variance
1.5	2.09 ± 0.11 (5.16)	2.06 ± 0.19 (9.01)
1	1.17 ± 0.09 (7.89)	1.22 ± 0.10 (8.16)
0.5	0.41 ± 0.04 (8.94)	0.50 ± 0.04 (7.47)
0.1	0.10 ± 0.01 (10.7)	0.10 ± 0.01 (11.5)

Values in parentheses are coefficients of variation (%).

exceed 10.8% even at the LOQ (i.e., 0.05 ng/ml) (Table 2).

3.5. Determination of fentanyl in swine serum

In human studies, transdermal fentanyl delivery systems with a nominal delivery rate of 75 μg/h were applied on patients undergoing surgery and the absorption characteristics of fentanyl were described by a zero-order process [23]. Plasma drug concentrations were determined to be near 1 ng/ml. In our case, the assay procedure developed was utilized to determine fentanyl concentrations in swine serum. Serum samples were collected from the animals at various time intervals following the application of a transdermal fentanyl delivery system with a nominal delivery rate of 50 μg/h drug for 72 h. A representative serum concentration vs. time plot is shown in Fig. 4. The serum fentanyl concentrations increased during the first eight hours after application of the fentanyl patch, reaching a peak value of 0.67 ng/ml. After that time, the drug concentration decreased gradually and remained detectable at 72 h (Fig. 4). Assuming a constant rate fentanyl input of 50 μg/h, an estimated value of the total body clearance (CL_{TB}) was calculated using the fentanyl dose delivered in 72 h divided by the area under the serum drug concentration vs. time curve between

Table 2
Accuracy of the assay and the variance of the calibration levels (n = 5 at each concentration)

Calibration level (ng)		Bias (%)	C.V. (%)
Added	Measured		
1.5	1.69	12.8	5.79
1	1.04	3.99	8.52
0.5	0.46	−7.09	5.88
0.2	0.18	−10.8	9.28
0.1	0.09	−6.57	3.57
0.05	0.05	6.16	10.8

Bias (%) = [(measured amount/added amount) × 100] − 100; (+)bias represents over-estimation; (−)bias represents under-estimation.

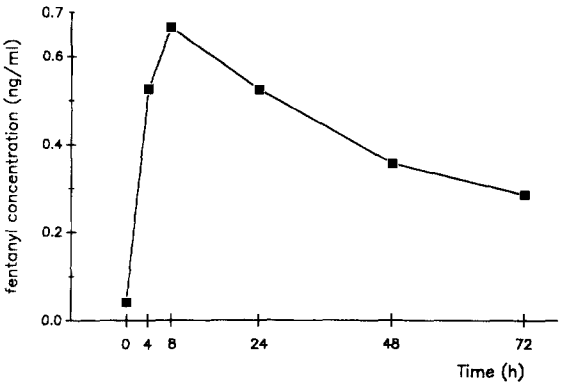

Fig. 4. A representative fentanyl concentration vs. time profile in the serum obtained from a swine following the application of a 50 μg/h fentanyl transdermal drug delivery system.

$t = 0-\infty$. In this manner, CL_{TB} was estimated to be 1.93 l h^{-1} kg^{-1}.

In conclusion, a sensitive and selective modified GC assay using MSD was developed for the quantitation of fentanyl in swine serum. Sample preparation is simple without the need for double-extraction and prior glassware deactivation procedures. The LOQ is 0.05 ng/ml (i.e., 2 pg at the detector) using 1 ml of serum. The assay demonstrates good reproducibility and is being applied to examine the pharmacokinetics of fentanyl in swine following transdermal drug administration.

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