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Note

High-performance liquid chromatographic determination of fentanyl citrate in a parenteral dosage form

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Fentanyl, N-(1-phenethyl-4-piperidyl) propionanilide, is a potent narcotic analgesic used in surgical anesthesia as the citrate salt at doses ranging from 2 to 50 μg/kg. As such, sensitive methods must be used for it's analysis in biological studies. These have included radioactivity measurement[1], radioimmunoassay (RIA)[2,3], high-performance liquid chromatography (HPLC)[4], gas chromatography (GC)[5] and GC–mass spectrometry and (GC–MS)[6]. Other studies have reported the characterization of fentanyl and related compounds by thin-layer chromatography (TLC), GC, GC–MS, IR and NMR[7–9] while two studies dealt with HPLC separations of fentanyl analogues[10,11]. More recent biological studies have included an HPLC–UV analysis of human plasma fentanyl[12] and a comparison between RIA and GC methods for fentanyl in human serum[13]. Few methods for fentanyl in pharmaceutical dosage forms have been published although the official methods for the drug substance and injection are titrimetric and GC respectively[14]. The present study describes an HPLC method for determination of fentanyl citrate injection at 50 μg base per ml and its application to prestability testing (*i.e.*, informal, short-term stress testing of drugs in dosage forms before formal stability testing).

EXPERIMENTAL

Apparatus

The HPLC system used consisted of a Waters Assoc. (Milford, MA, U.S.A.) 510 pump with a 1.0-ml/min flow, a 712 WISP autosampler with a 20-μl injection volume and an 820 data station. A Kratos (ABI Instruments, Ramsey, NJ, U.S.A.) 757 variable-wavelength UV–VIS detector was used at 229 nm with a sensitivity of 0.05 a.u.f.s. along with a Fisher (Springfield, NJ, U.S.A.) Recordall 5000 recorder and a Whatman (Clifton, NJ, U.S.A.) Partisil ODS-3, 25 cm × 4.6 mm I.D., 10 μm particle size column run at ambient temperature.

Reagents

Methanol and water were HPLC grade, 85% phosphoric acid, sodium hydroxide and 37% hydrochloric acid were reagent grade from Fisher (Fair Lawn, NJ, U.S.A.). Hydrogen peroxide (30%) was reagent grade from Mallinckrodt (St. Louis,

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MO, U.S.A.). Fentanyl citrate was from Johnson Matthey (West Deptford, NJ, U.S.A.) and Sublimaze®, control number 35C051 exp. 3-88 manufactured by Janssen Pharmaceutica (Piscataway, NJ, U.S.A.) was used.

The mobile phase consisted of water–methanol–85% phosphoric acid (500:500:4) which was filtered before use with a 0.45-μm polyvinylidene difluoride membrane filter (Durapore®, Millipore, Bedford, MA, U.S.A.).

Standard preparation

A solution was prepared in a 100-ml volumetric flask containing about 78.5 mg (accurately weighed) fentanyl citrate which had been previously dried at 60°C for 2 h and was diluted to volume with water. This solution was equivalent to about 0.500 mg fentanyl base per ml and was further diluted with water to 0.05 mg/ml fentanyl base.

Linearity of recovery

Samples were prepared containing 0%, 80%, 100% and 120% of the nominal 0.05 mg/ml fentanyl base level in duplicate in placebo. These were analyzed without further dilution.

Linearity of detector response vs. standard concentration

A stock standard at 0.500 mg/ml fentanyl base in water was serially diluted with water down to 0.005 mg/ml. These standards were assayed without further dilution.

Fentanyl citrate injection, Sublimaze injection and laboratory batch sample preparation

These samples were assayed directly without further dilution at the 0.05 mg/ml level.

Stress study samples

Solutions were prepare containing 0.125 mg/ml fentanyl base and either 3 *M* hydrochloric acid, 3 *M* sodium hydroxide or 15% hydrogen peroxide and heated 4 h in a 90°C water bath. These solutions were then cooled to room temperature, neutralized and diluted with water to 0.05 mg/ml. Similarly 0.125 mg/ml fentanyl base solutions were treated with a gentle stream of air or with 2000 lumens ft.$^{-2}$ of visible light at room temperature for 4 h and diluted to 0.05 mg/ml with water.

Calculations

The concentration (mg/ml), fentanyl ($C_{22}H_{28}N_2O$) was calculated using the relationship

$$\text{concentration} = \frac{R_u}{R_s} C\,(0.636)$$

where R_u and R_s are the peak response of the sample preparation and the response average of the two standards bracketing the sample respectively, C is the standard concentration in mg/ml fentanyl citrate and 0.636 is the conversion factor from fentanyl citrate to fentanyl base.

TABLE I

LINEARITY OF RECOVERY RESULTS

Fentanyl base added (mg)	*Peak height*		*Peak area*	
	Fentanyl base found (mg)	*Recovery (%)*	*Fentanyl base found (mg)*	*Recovery (%)*
0.00	0.00		0.00	
0.00	0.00		0.00	
2.00	1.99	99.5	1.98	99.0
2.00	2.00	100.0	1.98	99.0
2.50	2.50	100.0	2.49	99.6
2.50	2.47	98.8	2.49	99.6
3.00	2.98	99.3	3.02	100.7
3.00	3.02	100.7	3.03	101.0
Average recovery (%)		99.72		99.82
R.S.D. recovery (%)		0.663		0.851
Correlation coefficient		0.9992		0.9999
Slope		1.005		1.045
Intercept		−0.0192		−0.114

RESULTS AND DISCUSSION

Formulation of lab and scale-up batches of fentanyl citrate injection required prestability assays in order to measure the effect of formulation and processing variables. The HPLC method used was rapid and simple with no chloroform extraction as in the official USP procedure[14]. The low UV absorptivity of the compound, however, necessitated detection at 229 nm and use of undiluted samples at 0.05 mg base/ml. The linearity of recovery study from synthetic samples at 80–120% claim value was run to demonstrate precision and accuracy of the method. Results shown in Table I indicate excellent recoveries whether calculated using peak height or area measurements with accuracies from 99.5–100.5% and precision values, relative standard deviation (R.S.D.) of $< \pm 1.0\%$. The 2.50 mg 100% level represents that amount of fentanyl base present in the 50 ml validation batch volume. Linearity of the detector response–concentration relationship results are shown in Table II where cor-

TABLE II

LINEARITY OF DETECTOR RESPONSE–CONCENTRATION RELATIONSHIP

Fentanyl base concentration (mg/ml)	*Peak height (mm)*	*Peak area ($\mu V\ s \cdot 10^{-5}$)*
0.005	5.0	0.274
0.010	10.0	0.549
0.050	50.0	2.76
0.100	100.3	5.53
0.500	–	27.6
Correlation coefficient	0.9999	0.9999

TABLE III

STRESSED FENTANYL SAMPLE RECOVERIES

Conditions (for 4 h)	*Recovery (peak area) (%)*
3 *M* Hydrochloric acid (90°C)	46.6
3 *M* Sodium hydroxide (90°C)	100.7
15% Hydrogen peroxide (90°C)	0.3
Aeration (room temperature)	100.1
Light cabinet (2000 lumens $ft.^{-2}$, room temperature)	98.3

relation coefficients of 0.9999 were found for both peak height and area measurement. These curves spanned a one-hundred fold range in concentrations centered at the nominal value of 0.05 mg/ml.

Method and system precision data were obtained from replicate standard and sample analysis. A system precision R.S.D. of 0.64% was obtained by replicate standard injection which can be compared to the R.S.D. recovery of 0.36% for six replicate sample analyses. This indicates a higher method than system precision for that particular run. Normally the method precision has a R.S.D. value larger than the system precision by a factor of about 1.4 (see ref. 15). Precision of the method was also displayed by replicate analysis of six samples of the commercial product Subli-

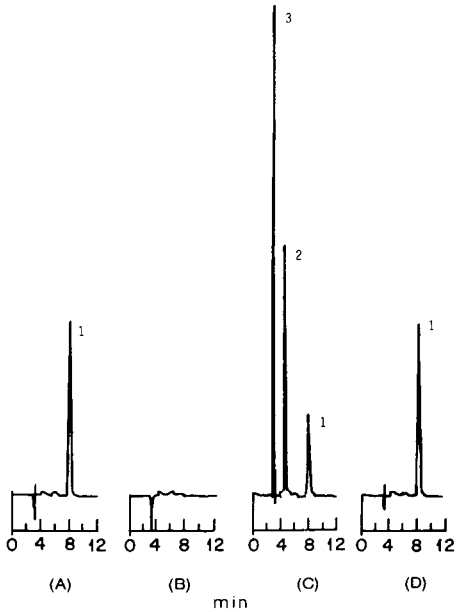

Fig. 1. Chromatograms of a fentanyl citrate standard preparation at 0.05 mg base/ml (A), placebo (B), 3 *M* hydrochloric acid stressed fentanyl sample (C) and unstressed fentanyl sample (D). Peak 1 is fentanyl in each chromatogram, and in (C) peaks 2 and 3 are a degradation product and the solvent peak respectively. Chromatographic conditions as in the text.

TABLE IV

PERCENT RECOVERY FENTANYL BASE–BATCH VOLUME RELATIONSHIP

Batch volume (ml)	*Recovery (peak area) (%)*
50	98.1, 99.7, 99.6
250	99.2*, 99.6*, 99.4*
1000	99.6, 99.6, 99.5*
10000	98.9*, 99.8*

* Samples assayed one day after preparation.

maze for fentanyl base all of which gave identical results by peak height or peak area.

Stability-indicating properties of the HPLC method were shown by the stress study recoveries listed in Table III. The compound was subject to acid degradation with a 46.6% recovery found after 4 h in 3 *M* hydrochloric acid at 90°C and was almost completely degraded by 15% hydrogen peroxide at the same time and temperature. Chromatograms of a standard preparation at 0.05 mg base/ml, a placebo, the 3 *M* hydrochloric acid stressed samples and an unstressed sample are shown in Fig. 1A–D respectively. A major unknown product of acid degradation was well separated from fentanyl with a 4-min retention time (peak 2 in Fig. 1C).

Scale-up of parenteral solutions from small lab batch volumes to large multiliter batch sizes can lead to variation in recovery of the active component with different manufacturing materials. When the fentanyl batch volume was increased from 50 ml to 10 l and assayed either on the day of production or the following day, recovery results shown in Table IV, fell between 98.1 and 99.8% indicating no interfering characteristics in the procedure.

The present HPLC method for fentanyl citrate was found to be rapid, suitable for analysis of the injectable dosage form and applicable to prestability studies. It is precise and accurate as shown by the linearity of recovery study, with a linear detector response–concentration relationship from 0.005 to 0.500 mg/ml injected. System and method precision were well within normal limits while the method showed stability-indicating properties and was appropriate for analysis of scale-up batches.

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