INTRODUCING A FULL VALIDATED ANALYTICAL PROCEDURE AS AN OFFICIAL COMPENDIAL METHOD FOR FENTANYL TRANSDERMAL PATCHES

[1,2]AMIR MEHDIZADEH, [1]TAYEBEH TOLIYAT, [1]MOHAMMAD REZA ROUINI, [3]FARZAD KOBARFARD

[1]Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, [2]Hakim Pharmaceutical Company, Tehran, [3]Department of Pharmaceutical Chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

ABSTRACT

A simple, sensitive and specific HPLC method and also a simple and fast extraction procedure were developed for quantitative analysis of fentanyl transdermal patches. Chloroform, methanol and ethanol were used as extracting solvents with recovery percent of 92.1, 94.3 and 99.4% respectively. Fentanyl was extracted with ethanol and the eluted fentanyl through the C18 column was monitored by UV detection at 230 nm. The linearity was at the range of 0.5-10 µg/mL with correlation coefficient (r^2) of 0.9992. Both intra and inter-day accuracy and precision were within acceptable limits. The detection limit (DL) and quantitation limit (QL) were 0.15 and 0.5 µg/mL, respectively. Other validation characteristics such as selectivity, robustness and ruggedness were evaluated. Following method validation, a system suitability test (SST) including capacity factor (k´), plate number (N), tailing factor (T), and RSD was defined for routine test.

Keywords: Fentanyl; Method validation; Transdermal patches; Drug release.

INTRODUCTION

Fentanyl, with an analgesic potency of about 80 times that of the morphine, was introduced into medical practice in the early 1970s for parenteral use in anesthesia. In 1991, a transdermal patch of fentanyl was approved for treatment of chronic and cancer pains (1). In spite of more than one decade of its marketing, no compendial method for analysis of fentanyl patches is available.

While there are two bioanalytical reports of GC-MS and GC-NPD, for determination of fentanyl in biological matrices and one monograph in USP 27 for Fentanyl Citrate Injection, no analytical method is reported for its transdermal patches (2-5). Several kinds of pressure sensitive adhesives (PSA) such as silicon type and also gelling polymer, hydroxyethyl cellulose (HEC), have been used in transdermal patches which may cause serious interferences in analytical procedures. The aforementioned bioanalytical methods are sensitive but these kinds of detectors are neither available in pharmaceutical quality control laboratories nor are common in finished product analysis.

The aim of the present study was to develop a simple, specific and sensitive analytical procedure for quantitative analysis of the fentanyl in patches and to provide data elements required for validation of assay category I, II, and III (6-8). Once the HPLC analytical procedure was developed and validated, SST was performed to evaluate the behavior of the chromatographic system, which is an integral part of any analytical procedures (9-12).

MATERIALS AND METHODS

Reagents, chemicals and instruments

HPLC grade acetonitrile and methanol and analytical grade glacial acetic acid and ammonium acetate were from Merck (Darmstadt, Germany). Fentanyl reference substance was purchased from European Pharmacopoeia. The HPLC systems which used were as follows: Waters 600 E pump, 487 UV detector, and Millennium integration data system (Waters, USA); Younglin Instrument, SDV 30 Plus, UV 730 D, and Auto Chrom data processor (Younglin, Korea); Philips PU4100 liquid chromatograph, PU4110 UV/VIS detector and PU4820 Series Chromate data system (Philips, UK).

Analytical Procedures

All analytical validation procedures for fentanyl transdermal patches were conducted by using Placebo Preparation or synthetic mixture (6 ,7).

Correspondence: Mohammad Reza Rouini, Faculty of Pharmacy, Tehran University of Medical Sciences, Faculty of Pharmacy, Tehran, 14155-6451, Iran. Email: rouini@tums.ac.ir

HPLC method: Fentanyl concentration in transdermal patches was determined by some mandatory modifications in the reported method (5) for its citrate salt in parentral dosage form. These modifications were alkalinization of the matrix and extraction of the fentanyl by a suitable solvent.

HPLC System: HPLC separation system consisted of a Capital C_{18}, (column 250×4.6 mm id, 5 μm); equipped with a guard column. The mobile phase consisted of 4 volumes of ammonium acetate solution (1 in 100) and 6 volumes of a mixture of methanol, acetonitril, and glacial acetic acid (400:200:0.6) which was adjusted to pH of adjusted to 6.6 ± 0.1 by addition of acetic acid. The flow rate of mobile phase was 1 mL per minute.

Preparation of Standard Curve: The solutions of 0.5, 2, 1, 3, 4, 5, 6, 8, and 10 μg/mL of fentanyl were prepared by spiking of PP at nine aforementioned concentrations. The concentration of *Test Solution* was 5 μg/mL, so the Analytical Validation Standard Solution (AVSS) covered from 10% to 200% of assay concentration.

Specificity: The specificity for assay was established by using the 3 sequential replicate of solution which was used in standard curve.

Accuracy: Accuracy was calculated as the percentage of recovery by the assay of the known amount of fentanyl which was added in the drug free patches. Two methods for determination of accuracy were performed; first, standard addition method, in which known quantities of fentanyl were added to PP, and second, commercial product method, in which known quantities of fentanyl were added to the commercial patches (6, 13, 14). In both methods, accuracy was assessed by at least 9 determinations for a minimum of 3 concentrations of fentanyl covering the specified range of 0.5-10 μg/mL.

Precision: The precision was demonstrated at three levels: repeatability (intra-assay or intra-day or within day Precision), intermediate precision (inter-day or between days precision), and reproducibility (between laboratories precision, collaborative studies). Each level of precision was investigated by 3 sequential replicate of injections of three concentrations of 0.5, 5 and 10 μg/mL. The precision was expressed as relative standard deviation (RSD) or coefficient of variation (CV). The HPLC system for intermediate precision and reproducibility were Philips PU 4100 liquid chromatograph and Younglin Instrument, SDV 30 Plus, UV 730 D, respectively.

Detection Limit (DL) and Quantitation Limit (QL): Ratios of 3:1 and 10:1 signal-to-noise were considered acceptable for estimation of the DL and QL, respectively (7).

Linearity and Range: The linear relationship was evaluated by three classical statistical methods, the correlation coefficient, y-intercept, and slope of the regression line. The linearity correlation coefficient (r^2), with/without QL should be more than 0.995.

Robustness: The robustness of the analytical procedure which is the measure of method's capacity to remain unaffected by small, but deliberate, variations in method parameters are shown in this study. Robustness (Table 1) was performed in 100% test concentration (5 μg/mL) and was explored using sample mixing time, mobile phase composition, mobile phase pH and flow rate.

Ruggedness: The ruggedness or the degree of reproducibility of the test results in different laboratories was demonstrated by the analysis of the same sample under different conditions of laboratories, analysts, instruments, lots of reagents, elapsed assay times, assay temperature and time. The HPLC system used for ruggedness study was Younglin Instrument, SDV 30 Plus, UV 730 D.

Analytical Procedures

Identification: The identification was performed as a part of *Assay*. The retention time of the major peak in the chromatogram of the *test solution*, in three sequential replicate of injections was compared to that of *standard solution*.

Assay: The protective liners equivalent to about 25 mg of fentanyl were removed from 10 reservoir patches. The rims of patches were carefully and completely cut and the opened patches were transferred to a 100-mL conical flask containing 50 mL ethanol and the pH of the mixture was adjusted to 9.5 ± 0.5 by addition of NaOH (0.01 N) solution. The flask was shaken for about 2 hours by mechanical means and then shaken twice for about 1 hour after addition of 25 mL of ethanol. A sufficient volume of mixture was centrifuged and 10 mL of supernatant liquid transferred into 100-mL volumetric flask and the volume was adjusted by addition of water. The final *Test Solution*, having fentanyl concentration of about 5 μg/mL, was prepared by dilution of 10 mL of this solution in a 50-mL volumetric flask with water.

Standard Solution: An accurately weighed quantity of Fentanyl Reference Standard (5 mg)

was transferred into 20-mL volumetric flask and dissolved in ethanol to obtain a stock standard solution having a known concentration of 250 µg/mL. The *Standard Solution*, having a concentration of about 5 µg/mL, was prepared by dilution of 1 mL of this solution in a 50-mL volumetric flask with water.

Equal volumes (50 µL) of the *Standard Solution* and the *Test Solution* were injected into the chromatograph. The quantity of fentanyl in each transdermal patch (in mg), was calculated by following formula:

$$\frac{C}{2} \times \frac{r_t}{r_s}$$

in which C is the concentration of fentanyl reference standard in *Standard Solution* (in µg/mL); and r_t and r_s are the fentanyl peak responses of *test* and *standard solution*, respectively.

Content Uniformity: After removal of release liners of ten fentanyl patches, their rims were carefully and completely cut and then each opened patch was separately transferred to a 50-mL conical flask containing 25 mL of ethanol and the mixture was shaken for about 2 hours by mechanical means and procedure was continued similar to assay section. The final test solution of about 5 µg/mL, was prepared by appropriate dilution with water.

Standard Solution: As described for *assay of standard solution* except that the amount of ethanol should be 10% v/v in content uniformity for standard solution.

Drug Release

Dissolution apparatus: Determination of fentanyl release pattern in transdermal patches was carried out by using USP 27 apparatus 5, paddle over disk, with the speed of 50 rpm. One patch was applied flat on the disk with the release surface facing up (effective area available to diffusion was 10 cm^2) and a spectra/por$^®$ 7 with cut off 14000 Daltons, was placed on its top as a support membrane. This membrane was rehydrated by immersion in purified water for 1 hour before application. At predetermined time intervals, 5 mL of samples were collected and immediately replenished with fresh medium. The samples were analyzed for their fentanyl content by HPLC method.

Dissolution medium: Water, PBS pH 7.2, water containing 5% methanol and 1% solution of sodium lauryl sulfate in water were equilibrated to 32 ± 0.5° C and evaluated.

RESULTS AND DISCUSSION
Analytical validation

Specificity: No peak was observed in the chromatogram of PPS at the retention time of fentanyl (Figures 1 and 2).

Accuracy: Accuracy was evaluated at three concentrations of 0.5, 5, and 10 µg/mL and the results are shown in Table 2.

The fentanyl extraction by chloroform, methanol and ethanol showed the recovery percent of 92.1, 94.3 and 99.4%, respectively. Therefore, ethanol was chosen as the best solvent for extraction in this study.

Precision: The results of three levels of precision, repeatability, intermediate precision and reproducibility are shown in Table 3. The highest RSD for all three levels of precision were observed for the lowest concentration (0.5 µg/mL) which are 1.14. 1.32 and 0.85% respectively

Detection and Quantitation Limits: The limit of detection and limit of quantitation were determined to be 0.15 and 0.5 µg/mL, respectively.

Linearity and Range (Calibration Curve): The summary of linearity parameters are shown in Table 4 and Fig. 3. As demonstrated in Fig. 3, the calibration curve covers concentrations of 10 to 200% of the test concentration (0.5 to 10 µg/mL).

Robustness: Ten percent deviation in pH, composition, and flow rate of mobile phase were chosen to show robustness. The results of robustness are summarized in Table 5. It should be mentioned that percent recovery and RSD under analytical procedure were 100.3 and 0.89, respectively.

System Suitability Test: On the basis of results of this analytical validation, the values for N, T, K´ were calculated. The capacity factor, k´, which should be more than 3 was found to be 3.52 for fentanyl. The column efficiency which usually should be more than 1000 theoretical plates, was determined to be 2048 for fentanyl. The tailing factor was determined as 1 which is less than usual pharmacopoeial standard (less than 2.0). The relative standard deviation for replicate injections was also less than 2.0%.

The solubility of fentanyl in three different dissolution mediums, water containing 5% methanol and 1% sodium lauryl sulfate solution, PBS (pH 7.2) and water was determined to be more than 2000, 431 and 53 µg/mL respectively.

The stability of the test solution was checked up to 48 h and it was found that it was stable up to 24 h. It was also found that the selection of injection

Table 1. Robustness changes in HPLC variable parameters.

Variable Parameters	Changing Range
Sample mixing time	2 hours ± 10%
Mobile phase composition	(400:200:0.6) ± 10%
pH of mobile phase	6.6 ± 10%
Flow rate of mobile phase	1 mL ± 10%

Table 2. Accuracy of the method in the range studied.

Acceptance criteria	Results	
	Concentration (µg/mL)	%Recovery
Accuracy (%recovery) should be between 95.0 and 105.0%.	0.5	99.4
	5	100.3
	10	100.1

Table 3. The precision of the method in the range studied.

Precision	Acceptance Criteria	Results		
		3 × 3 method		
		Concentration (µg/mL)	RSD of Peak area	RSD of R.T .
Repeatability (intra-day precision)	The RSD of peak areas and retention time of fentanyl in the *AVSS* should not be more than 2%.	0.5	0.87	1.14
		5	0.54	0.95
		10	0.45	0.74
		1 × 6 method		
		5	0.78	0.58
Intermediate precision (inter-day precision)	The RSD of peak areas and retention time of fentanyl in the *AVSS* should not be more than 2%.	**3 × 3 method**		
		0.5	0.65	1.32
		5	0.89	0.96
		10	0.45	0.68
		1 × 6 method		
		5	1.08	1.4
Reproducibility (inter-laboratory precision)	The RSD of peak areas and retention time of fentanyl in the *AVSS* should not be more than 2%.	**3 × 3 method**		
		0.5	0.74	0.85
		5	1.25	0.74
		10	0.61	0.42
		1 × 6 method		
		5	0.55	0.84

RSD: relative standard deviation; AVSS: Analytical Validation Standard Solution

Table 4. Regression line parameters for fentanyl analytical procedure.

Linearity parameters	Results
Correlation coefficient	0.9992
Slope of the regression line	16686
Y-intercept	2419

Table 5. Summary of robustness results for fentanyl analytical procedure

Robustness Changes	Acceptance criteria	Results
Sample mixing time	%Recovery: 95-105	99.5
	RSD of peak area < 2%	0.48
Mobile phase composition	%Recovery: 95-105	99.2
	RSD of peak area < 2%	1.21
pH of mobile phase	%Recovery: 95-105	101.7
	RSD of peak area < 2%	1.24
Flow rate of mobile phase	%Recovery: 95-105	99.4
	RSD of peak area < 2%	0.68

RSD: relative standard deviation
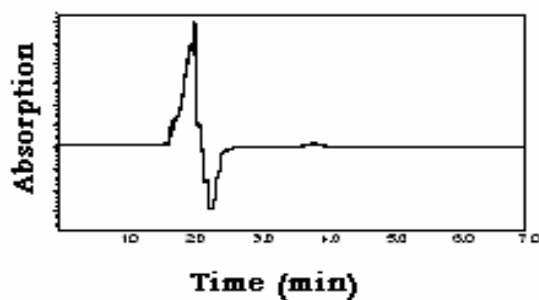
Figure 1. Chromatogram of placebo solution. There is not any peak at the retention time of fentanyl.
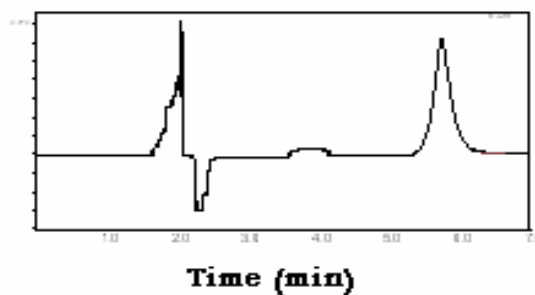
Figure 2. Chromatogram of Analytical Validation Standard Solution. There is not any interfering peak around the retention time of fentanyl.

$$y = 16.686x + 2.419$$
$$R^2 = 0.9992$$
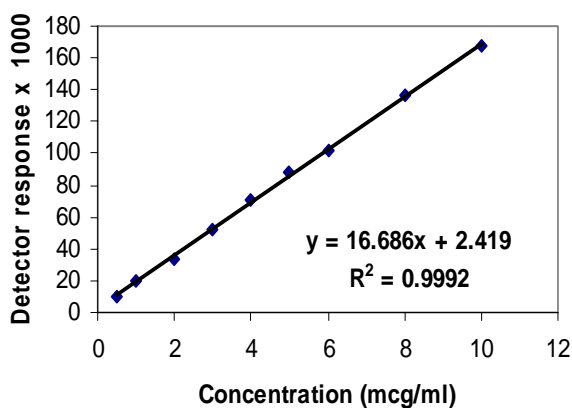
Figure 3. Calibration curve for fentanyl solution.
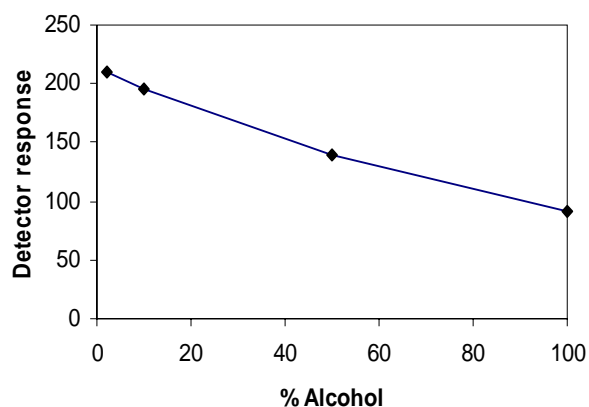
Figure 4. Influence of percent of ethanol on sensitivity of detector.

solvent for fentanyl sample is very critical. Increasing the percentages of ethanol in injection solvent decreased the plate number and sensitivity of detector and caused peak broadening. The number of theoretical plates and the sensitivity of detector were decreased from 2400 to 600 and from 210 to 92 respectively when ethanol was increased from 2 to 100% in injection solvent (Fig. 4). This phenomenon could be due to some delay in partitioning of fentanyl between solvent and stationary phase in the HPLC column.

According to our results, the solubility of fentanyl in water containing 5% methanol and 1% sodium lauryl sulfate in water was much more than sink condition. This very high solubility may lead to the loss of the discriminative property of dissolution medium. On the other hand, the solubility of fentanyl in water was such low that using fentanyl patches with strengths higher than 25 µg/h may result in loosing the sink condition. As a result, a 500 ml of PBS with the pH of 7 equilibrated to 32 ± 0.5 ºC was considered to be a suitable medium for dissolution of fentanyl patches.

CONCLUSIONS

An HPLC method for analysis of fentanyl in patches was developed which covers all the requirements of validation (specificity, accuracy, intra-day and inter-days precision, reproducibility, DL, QL, linearity). A simple procedure for extraction of fentanyl from patches and also the application of the analytical method to prepare a compendial method for fentanyl patches are presented.

REFERENCES

1. Barry WB. Novel mechanism and devices to enable successful transdermal delivery. Eur J Pharm Sci 2001; 14: 101-114.
2. Choi HS. Quantitative analysis of fentanyl in rat plasma by gas chromatography with nitrogen–phosphorus detection. J Chrom B 2001; 765: 63–69.
3. Portier EJG, Blok K, Butter JJ. Simultaneous determination of fentanyl and midazolam using high performance liquid chromatography with ultraviolet detection. J Chrom B 1999; 723: 313–318.
4. Yuansheng L, Yutian WU, Jing Z. Capillary GC Determination of fentanyl and midiazolam in human plasma. Mic Chem J 1996; 53: 130–136.
5. United States Pharmacopeia 27, Rockville, MD. 2004.
6. International Conference on Harmonization, ICH. Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures. Geneva 1996.
7. US FDA, Technical Review Guide Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), Rockville, MD 1998.
8. General Chapter <1225>, Validation of compendial methods. United States Pharmacopeia 27, Rockville, MD 2004.
9. McDowall RD. The role of laboratory information management systems (LIMS) in analytical method validation. Anal Chim Acta 1999; 391: 149-158.
10. Ivanoic D, Medenica M, Malenovic A, Jancic B. Validation of the RP-HPLC method for analysis of hydrochlorothiazide and captopril in tablets. Accred. Qual. Assur. 2004; 9: 76-81.
11. Huber L. Qualification of high performance liquid Chromatography systems. J BioPharm 1998; 11: 41-44.
12. Winslow PA, Meyer RF. Defining a master plan for the validation of analytical methods. J Valid Tech 1997; 14: 361-367.
13. Hokanson GC. A life cycle approach to the validation of analytical methods during pharmaceutical product development, part I: The initial validation process, Pharm Tech 1994; 21: 118-130.
14. Renger H, Fischer J M, Funk W. Validation of analytical procedures in pharmaceutical analytical chemistry: HPTLC assay of theophylline in an effervescent tablet. J Planar Chrom 1995; 8: 269-278.
15. Burgess C. Development and validation of analytical methods. Anal Chem Acta 1997; 338: 163-165.
16. Ellison SLR, Williams A. Measurement uncertainty and its implications for collaborative study method validation and method performance parameters. Accred Qual Assur 1998; 3: 6-10.
17. van Zoonen P, Hoogerbrugge R, Gort SM, van de Wiel H. Some practical examples of method validation in the analytical laboratory. Trend Anal Chem 1999; 18: 584-593.
18. Lauwaars M. Methods validation: AOAC's three validation systems. Accred Qual Assur 1998; 3: 32-35.