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DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FENTANYL CITRATE BOTH IN BULK AND MICRO-EMULSION FORMULATION

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ABSTRACT: Objective: Development of simple, rapid, and accurate UV spectrophotometric method for the estimation of Fentanyl citrate (FC) in a bulk and pharmaceutical dosage form. The proposed method was validated according to the guidelines of the International Conference on Harmonization and the Association of Official Analytical Chemists International. **Methods:** The method was based on the measurement of absorbance at a wavelength of257.6 nm, λ_{max} of FC in methanol, respectively. **Results:** The calibration curve of FC was linear in the concentration range of 200-1400 µg ml^{-1}. The mean recovery, the limit of quantification (LOQ) and limit of detection (LOD) for FC were 99.029%, 1.031 µg ml-1and 0.340 µg ml^{-1}, respectively. The method was precise, with a relative standard deviation of less than 2.0 %. For robustness, the factors analyzed did not significantly affect the quantification of FC. **Conclusion:** The proposed method can be successfully applied for the estimation of FC in a bulk and pharmaceutical dosage form.

INTRODUCTION: Fentanyl citrate (FC) is chemically known as N - phenyl - N - [1 - (2 - phenylethyl) - 4 - piperidinyl] -, 2 – hydroxyl - 1, 2, 3 - propanetricarboxylate (1:1) [1, 2, 3, 4]. The molecular formula of FC is $C_{22}H_{28}N_2O$, $C_6H_8O_7$ and the molecular weight is 528.59 **Fig. 1**. It is a potent synthetic opiate analgesic with a potency of 50-100 times higher than that of morphine [5]. Their main therapeutic effects are analgesia, sedation, and attenuation of responses to potent sympathetic stimuli.

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As an analgesic, it has been used successfully by intravenous or sublingual routes for the treatment of neuroleptic analgesia and sedation during preoperative, induction, maintenance, and post-operative surgical periods. FC, opioid narcotic analgesic available in various dosage forms in US and EU market.

A few examples of dosage forms of FC currently available in the market in different dosage forms by their trade names are Abstral® and Fentora® sublingual tablet; Fentanyl and Fentora® buccal tablet; Fentanyl citrate injection and Breakyl® buccal film [6]. A detailed survey of literature revealed that very few analytical methods had been reported for the estimation of FC in dosage formusingHigh Performance Liquid Chromato-graphy (HPLC) [7, 8, 9, 10]. Not even a single spectrophotometric method has been reported for

estimation of FC in bulk or pharmaceutical dosage form. An analytical method has also been described for the estimation of FC in biological fluids using HPLC [11, 12] and GC-MS method [13]. No spectrophotometric analytical method has yet been reported in the official compendia for the determination of FC in pharmaceutical dosage forms. The present study attempted to develop a rapid, economical, precise and accurate method for determination of FC. The UV spectrophotometric method permits analysis of the drug without previous separation and extraction procedures. This alternative is simpler and less expensive than HPLC methods [7, 8, 9]. The objective of the present study was to develop a simple, precise, accurate and rapid UV spectrophotometric method for the estimation of FC in a pharmaceutical dosage form.

MATERIALS AND METHODS:

Apparatus: Shimadzu 1800 UV–Vis double beam spectrophotometer with UV probe software and 1 cm matched quartz cells was used.

Materials: Fentanyl citrate USP (FC) was procured fromRusan Pharma Ltd. Works, Ankleshwar, Gujrat. Analytical grade methanol (Merk Ltd.) was used throughout these experiments.

Standard Solution: Stock solutions of FC was prepared by dissolving 100.0 mg of FC in methanol in 50 ml volumetric flask and sonicated for 5 min. Final volume of the solution was adjusted with methanol to get stock solution with the concentration of 2000.0 µg ml^{-1} of FC.

Working Standard Solutions: Working standard solution of FC was prepared by pipetting 2.0 ml of FC standard solution of 2000.0 µg ml^{-1} in a 10 ml volumetric flask. The final volume of the solution was adjusted with methanol to get a working standard solution with a concentration of 400.0 µg ml^{-1} of FC. The concentration range of 200, 400, 600, 800, 1000, 1200, 1400 µg ml^{-1} was prepared for FC from standard solution for determination of the linearity range.

Sample Solution: The accurately weighed quantity of ME containing equivalent to about 8.0 mg of FC was accurately weighed and transferred to 20 ml of volumetric flask. Approximately 10 mL of methanol was added to the flask, and the contents were sonicated for 5 min, and then the final volume was made up with methanol. The final concentration of 400 µg ml^{-1} of FC was obtained, and the blank was prepared in a similar manner.

Method Development:

Selection of Wavelengths: The solution of FC (400 µg ml^{-1}) was scanned from 220 to 400 nm with the UV spectrophotometer using methanol as a blank. The UV spectrum of FC [14, 15] was shown in the **Fig. 1**; thus the three λ_{max} of FC was found to be 251.4 nm, 257.6 nm and 263.4 nm in methanol, respectively & the maximum absorbance was obtained at the λ_{max} of 257.6 nm with respect to other two wavelengths.

Validation of the Concentration Range: The absorbance was measured for FC in the concentration range of 200, 400, 600, 800, 1000, 1200, 1400 µg ml^{-1} at 257.6 nm. Calibration graph was plotted for FC at 257.6 nm at a concentration range of 200-1400 µg ml^{-1} as shown in **Fig. 2**.

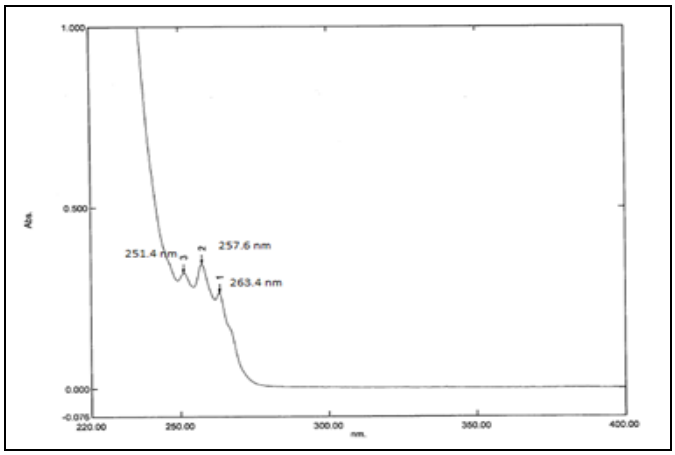

FIG. 1: UV SPECTRA OF FENTANYL CITRATE

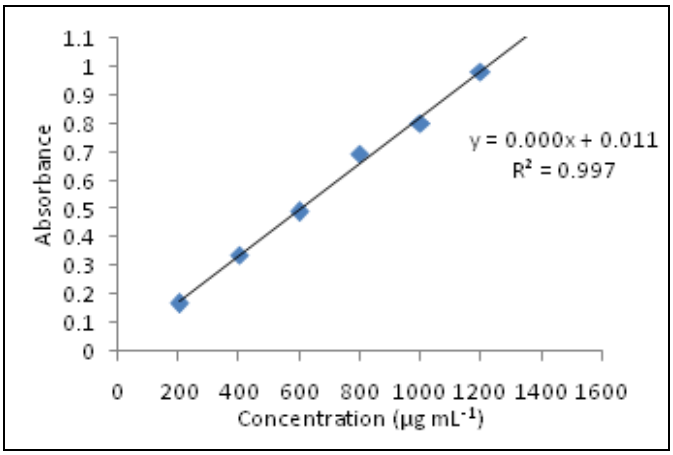

FIG. 2: CALIBRATION GRAPH FOR FENTANYL CITRATE

Method Validation: Validation of this spectrophotometric method was carried out as recommended by the International Conference on Harmonization ICH (ICH Q2 (R1)) guidelines[16]and the Association of Official Analytical Chemists International[17]for the parameters of specificity, linearity, accuracy, precision, the limit of detection (LOD), limit of quantification (LOQ) and robustness.

Specificity: The specificity of the method was evaluated through analysis of a placebo solution. The mixture of inert components was prepared in the usual concentrations employed in developed FC microemulsion. These solutions were analyzed by the proposed method in order to determine if any of the components of the formulation might affect the determination of FC.

Linearity: Linearity was determined from the calibration graph plotted for FC at 257.6 nm. All spectrophotometric determinations were performed in triplicate and at room temperature (25±2 ºC). The linear regression was calculated by the method of least squares, and the calibration curve was evaluated by analysis of variance (ANOVA).

Accuracy: The accuracy was calculated based on the percentage of recovery of the known amounts of FC added to the samples [16]. Aliquots of the FC standard solutions in concentrations of 600, 800 and 1000 µg ml^{-1} were transferred to 10 ml volumetric flasks containing 0.3 ml of sample solution. The volumes were made up with methanol and the drugs were determined in triplicate, using the proposed method.

Precision: The intra-day precision (repeatability) was evaluated by analyzing sample solution at single concentrations of FC. The analyses were performed in triplicate on the same day. To estimate the inter-day precision, the sample solution was freshly prepared at the same concentration level, and the response was determined in triplicate. This procedure was performed on three consecutive days. The intra- and inter-day precisions are expressed in terms of relative standard deviation (%RSD).

Limits of Detection and Quantitation (LOD & LOQ): LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve. The standard deviation of y-intercepts of regression lines was used as the standard deviation of responses [16]. These values were obtained using the following equations:

$$LOD = 3.3\ \sigma / S \qquad \text{(Eq. 1)}$$

$$LOQ = 10.0\ \sigma / S \qquad \text{(Eq. 2)}$$

Where, σ = the standard deviation of the response. S = the slope of the calibration curve

Robustness: The robustness study was carried out by changing the wavelength in ± 1 nm of 257.6 nm.

RESULTS AND DISCUSSION:

Method Development: FC is highly soluble in methanolhenceit is used as a solvent for standard and sample preparation. The three λ_{max} of FC was found to be 251.4 nm, 257.6 nm, and 263.4 nm in methanol, respectively & the maximum absorbance was obtained at the λ_{max} of 257.6 nm with respect to the other two wavelengths. The absorbance values were taken at 257.6 nm for FC, and then the calibration curve was plotted at the respective wavelength as presented in **Fig. 2.**

Method Validation:

Specificity: The specificity test demonstrated that excipients of the microemulsion affect the drug determination at 251.4 nm as depicted in Fig. 3; because of the interference of the excipients at λ_{max} of 251.4 nm. Further, the specificity test demonstrated that the excipients did not affect the drug determination at 257.6 nm, as depicted in **Fig. 3**. Thus, no interference was observed at 257.6 nm indicated that the method is specific.

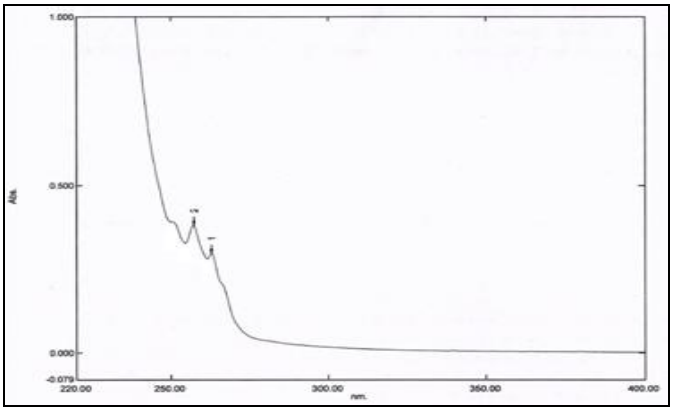

FIG. 3: GRAPHICAL EVIDENCE THAT NO INTERFERENCE EXISTS BETWEEN THE FENTANYL CITRATE AND EXCIPIENTS OF DEVELOPED MICROEMULSION AT λ_{max} OF 257.6 nm

Linearity: The statistical result of the linear regression for FC is shown in **Table 1**. The coefficient of determination indicated good linearity *i.e*. 0.9977 for FC.

The linearity range was 200-1400 µg ml^{-1} for FC. The absorbance values for these concentration ranges remained between 0.1 and 0.9, conforming to the recommendations of Vogel [18].

The HPLC method for the estimation of FC in pharmaceutical dosage forms shows a smaller range of concentrations [7, 9].

TABLE 1: STATISTICAL AND VALIDATION PARAMETER

Statistical Parameters	FC
λ_{max}	257.6 nm
Linearity range (µg ml^{-1})	200 - 1400
Regression equation (y = mx + c)	Y=0.0008x+0.011
Slope (m) Intercept (c)	0.00080.011
Coefficient of determination (R^2)	0.9977
Significant slope (p -value[a])	< 0.0001
LOD (µg ml^{-1})	0.340
LOQ (µg ml^{-1})	1.031

λ_{max}- maximum wavelength, [a] Theoretical value of *p* is based on one – way ANOVA test at α = 0.05 level of significance

Accuracy: The recovery percentage was 99.029% along with % RSD 0.121for FC **Table 2**. This result indicated the accuracy of the method.

TABLE 2: RECOVERY DATA FOR STANDARD SOLUTIONS ADDED TO THE SAMPLES ANALYSED BY UV SPECTROPHOTOMETRIC METHOD

Drug	Theoretical Concentration (µg ml^{-1})	Experimental Concentration Found[a] (µg ml^{-1})	% RSD	Recovery (%)	Mean Recovery (%)	% RSD
FC	600	604.58	0.119	100.76	99.029	0.121
	800	784.58	0.092	98.07		
	1000	982.50	0.127	98.25		

RSD - relative standard deviation a mean of 3 determinations

Precision: The precision parameters (%RSD) expressed as repeatability (intra-day) and as intermediate precision (inter-day) are presented in **Table 3**. For FC, all % RSD were values lower than 1% for repeatability in analyses performed during 3 consecutive days and also for intermediate precision. Thus, the proposed method has good precision in the spectrometric determination of FC.

TABLE 3: INTRA DAY AND INTER DAY PRECISION RESULTS

Parameters	Sampling time	FC				
		Amount Present (mg)	Amount Present (%)	Mean Amount Present (%)	SD	RSD (%)
Intraday precision	0 h	0.967	99.66		0.074	0.074
	4 h	0.975	100.47	100.21		
	8 h	0.971	100.09			
Inter day precision	Ist day	0.971	100.09		0.746	0.746
	IInd day	0.968	99.79	99.99		
	IIIrd day	0.971	100.09			

SD - standard deviation and RSD - relative standard deviation

Limits of Detection and Quantitation (LOD & LOQ): The LOD was 0.340 µg ml^{-1} and the LOQ was1.031 µg ml^{-1} for FC, respectively. These values show that the proposed method has good sensitivity, and results are presented in **Table 1**.

Robustness: The responses of FC did not change significantly when the analytical conditions were modified **Table 4.** These observations confirm the robustness of the method for the determination of FC in the pure and pharmaceutical dosage form.

TABLE 4: RESULTS OBTAINED BY CHANGING THE WAVELENGTH ± 1 NM

Wavelength (nm)	FC		
	Amount Present (mg)	Amount Present (%)	RSD (%)
258.6	0.905	95.31	0.080
256.6	0.929	97.81	0.078

RSD - relative standard deviation

The objective of this study was to develop and validate a simple & specific UV spectro-photometric method for determination of FC. The absorbance of FC was maximum *i.e.,* 0.341 at λ_{max} of 257.6 nm with respect to an absorbance of 0.262 and 0.315 at λ_{max} of 263.4 nm & 251.4 nm, respectively. Hence, the fourth UV spectro-photometric estimation of FC was carried out at

λ_{max} of 257.6 nm using methanol as blank. This method exhibited precise, accurate, and cost-effective assay for FC in microemulsion formulation and other pharmaceutical dosage forms. Based on previous studies, Mehdizadeh A *et al*., (2005) developed a fully validated analytical procedure as an official compendial method for fentanyl transdermal patches [7]. The developed method was linear in the range of 0.5 – 10 µg $ml^{-1,}$ and the recovery study for this compound was 94.3%. Piekarshi M *et al.,* (2012) developed and validated an HPLC method to determine the stability of fentanyl citrate and bupivacaine hydrochloride mixtures in infusion solutions [9]. The developed method was linear in a range of 2.5 - 7.25 µg mL^{-1} for FC with LOD and LOQ values of 0.5 µg mL^{-1} and 1.5 µg mL^{-1}, respectively [9].

Almousa A *et al.,* (2011) developed the HPLC-UV method for the determination of fentanyl in rat plasma and its application to elucidate pharmacokinetic behavior after *i.p.* administration to rats. The method was linear in the range of 5.0 - 100 ng mL^{-1} & for precision study; the RSD values were less than 12.6% [11]. Literature survey revealed that none of the spectrophotometric methods was available for the determination of FC in raw material or in pharmaceutical formulations. Though the above-mentioned processes for the estimation of FC are more sensitive than a present method, however all the previous methodologies involved the use of highly sensitive apparatus like HPLC & hyphenated instruments such as GC-MS along with costly organic solvents required for mobile phase development as compared to UV spectro-photometer as employed in this study. The present method using UV spectrophotometer is more cost-efficient & less time-consuming.

CONCLUSION: A UV spectrophotometric method was developed and validated for the determination of FC in bulk and pharmaceutical dosage formulations. The method showed precision, accuracy, LOD, LOQ, and robustness, as evaluated according to ICH guidelines. The proposed method proved to be simpler, less expensive and faster because no additional pre-treatment of the samples is required prior to the measuring step, thus accelerating the quality-control process. Thus the UV spectrophotometric method was suitable, useful, and an excellent alternative to HPLC to assess quality in routine analysis of FC in drug products.

Permission: License was granted to M/s Zim Laboratories Ltd. by food & Drug Administration M.S. for working with Narcotic Drugs & Psychotropic Substances.

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