DOI Number: 10.5958/0973-9130.2019.00171.3

Simple and Sensitive Technique for Fentanyl Quantification in Urine and Plasma by GC-MS

Priyamvada Sharma[1], Bharat Venkatesh[2], Pratima Murthy[3], Manoj Kumar Sharma[4]

[1]Senior Scientific Officer, [2]Junior Scientific Officer, [3]Professor , Centre for Addiction Medicine, Department of Psychiatry, [4]Professor of Psychology, Department of Psychology, National Institute of Mental Health and Neurosciences (NIMHANS), No. 2900, Hosur Road, Bangalore, India

Abstract

This work reports a sensitive and specific gas chromatography (GC) mass spectrometry (MS) method for the detection of subnanogram concentrations of fentanyl in plasma.

Objective- Fentanyl is a high-potency, rapid–onset synthetic opioid prescribed for the treatment of chronic pain and used as a surgical anesthetic. In the past decade its abuse and accidental overdose have steadily been on the rise.

Method- This study was planned to develop an analytical procedure for fentanyl detection in biological samples which will help in to distinguish between therapeutic drug use and abuse. For sample preparation Solid phase extraction coupled (SPE) was opted while quantification was done with Gas Chromatograph Mass Spectrometer (GC-MS). GC-MS is among the best known precise and effective techniques for biological samples analysis. Chromatographic separation was achieved on using DB-5MS column on a selected ion monitoring (SIM) mode.

Results-Signal to noise ratio was 3:1.The linearity lay between 0.62-40ng/ml correlation coefficient was ≥ 0.9899 and recovery was ≥80%. Limit of Detection was 0.6ng/ml and limit of quantification was 0.24ng/ml.

Conclusion- Current analytical procedure for fentanyl quantification had good specificity and sensitivity and showed good recovery. This method will be helpful for quantification of fentanyl in clinical and can be applied to forensic as well .

Keywords*: Gas chromatography, Mass Spectrometry, Solid phase extraction*

Introduction

Fentanyl, a potent analgesic drug, has traditionally been used intravenously in invasive or diagnostic operations. Formulations with fentanyl in oral transmucosal delivery system and in transdermal depot-patch have also been developed against breakthrough pain in cancer patients[(1).] This synthetic narcotic analgesic is of high potency with short duration of action and it is more potent than morphine. Fentanyl has a high safety margin if taken in prescribed format. But Fentanyl can be abused and is subject to criminal diversion [(2).] Mixing fentanyl with street-sold heroin or cocaine markedly amplifies potency and potential dangers. It produces significantly worse respiratory depression and non-medical use of fentanyl has resulted in numerous deaths [(3, 4).] Accidental overdose and abuse of anesthetic agents is a matter of concern. Cases of fentanyl over use have increased from 3 in 2000 to 12 in 2003 in United States [(1).] In India accidental overuse has claimed precious lives recently (The Hindu, 15 Feb2013). Propofol, midazolam, ketamines and fentanyl are commonly used for anesthesia [(2)] among which fentanyl is a synthetic narcotic analgesic 80-100times

Corresponding Author:
Dr. Priyamvada Sharma, PhD
Senior Scientific Officer, CAM, NIMHANS,
Bangalore-560029, India
E-mail: ps842010@gmail.com

more potent than morphine which make it potential for abuse [3]. Although, it is chemically unrelated to opiates, (4) it exhibits similar pharmacological & toxicological actions including analgesia, euphoria, respiratory depression and physiological dependence similar to opiates [5]. The therapeutic popularity has not been without problems. As a potent narcotic, fentanyl abuse is a known problem among health professionals. Recreational abuse is extremely dangerous [6] and needs to be included in clinical and forensic toxicology investigation, which requires fast, precise and accurate measurements.

The Centers for Disease Control and Prevention (CDC) has released a report regarding the increase in the number of illicit drug overdose deaths, potentially due to the use of acetyl fentanyl [5]. Acetyl fentanyl is a fentanyl analog previously undocumented in illicit drug use and is not available as a prescription drug in the U.S. The CDC recommends to laboratories to exercise increased vigilance in the detection of this substance (immunoassay). Fentanyl is extensively metabolized, blood concentration of 1-3ng/ml is sufficient for analgesia and 4-10 ng/ml for anesthesia during surgery and only 0.4 -6% of the dose is excreted unchanged in urine [6,7]. This makes it difficult to analyze the drug in body fluids.

Existing literature contains little data concerning urine and plasma concentrations of fentanyl in terms of recreational use in our population. Although screening by immunoassay can detect its use over the prescribed cut off. Some Chromatography techniques along with Mass -Spectrometer are able to confirm the exact quantity. A highly sensitive and specific technique that can quantitate fentanyl up-to subnano levels is thus essential. Detection and quantification of fentanyl may prove beneficial for patient care, but it requires sensitive and specific analytical proof. Since therapeutic levels of fentanyl are as low as one ng /ml in plasma methods with high sensitivity are required for fentanyl determination.

GC-MS offers the best sensitivity among the currently available methods for determining fentanyl in biological fluids in the Single Ion Monitoring (SIM) mode of operation, which affords unparalleled specificity. Thus Gas chromatograph Mass Spectrometer (GC-MS) technique for quantification was selected. The objective was to develop a method for quantification of fentanyl in plasma and urine which can be used in our population where recreational use of fentanyl is suspected. This work is the first step towards detection and quantification of anesthetics in the clinical and forensic situation and will help to distinguish therapeutic use and abuse.

Material and Method

Population- The study was carried out over a period from April 2016 to Dec 2017 attending Centre for Addiction (CAM) Out Patient Department of National Institute of Mental Health and Neurosciences, Bangalore, India. All subjected with suspected fentanyl use were selected for the study and their biological samples were collected with consent .All samples were labeled and stored in -80°C till analysis. Ethical approval was obtained.

Standards and Reagents- All chemicals used were of analytical grade. Bulk solvents and routine chemicals were obtained from Sisco Research Laboratories (Mumbai, India) and Merck & Co. Inc (Whitehouse Station, NJ, USA). Fentanyl standard were procured from Cerilliant Corporation, Round Rock, Texas. Initial fentanyl stock solution of 1000ng/ml was prepared in double distilled water and working concentrations were made from this. The standards were protected from light and stored in -20°C until analysis. For extraction of biological samples bond elute solid phase cartridges were purchased from (Agilent Technologies (USA).

Sample Collection and Preparation: Small volume of 1-3 ml of biological sample (plasma, urine) were collected and kept 2-8° C immediately, later stored at -80° C till analysis. Urine samples were first screened with Abon screening cassettes. Samples positive by fast screening were further quantified, since for plasma samples screening device are unavailable so all samples were quantified using GC-MS.

For sample preparation 1-3 ml of biological sample was mixed with 100mM Phosphate buffer (pH-6.0) in 1:2 ratio, 100mM diabasic Sodium phosphate was added to adjust pH 6.0 ±0.5. Solid phase extraction was carried out using bond elute cartridges. Column was conditioned with Methanol, distilled water and 100mM Phosphate buffer (1:1:0.33). Sample was applied at the rate of one ml per minute followed by washing with distilled water, 0.1M Acetic acid and Methanol (1:0.33:1) v/v. After drying elute was collected in Dichloromethane/ Isopropyl alcohol/Ammonia (78:20:2) (pH 11-12). Evaporation was completed using Genevac EZ-2

series vacuum evaporators. Genvac low boiling point liquid programme ensures absolute and desiccated evaporation. These dry pure samples were reconstituted 50.0 µL of ethyl acetate.

Instrumentation- For identification and quantification of extracted samples Agilent 7890A series GC interfaced with 5975C quadrapole MS (Agilent technologies, Santa Clara, CA) was applied. The quantification was performed in the selected–ion monitoring (SIM) mode using DB-5MS fused silica capillary column (122-5532G) carrier gas was helium at flow rate of 1ml/min. Data processing was done with HP chemstation software. Injection was made by Agilent 7693 auto sampler in split-less mode. The injection port temperature was maintained 270°C and ion source temp was 230°C the electron multiplier voltage was 70 ev. Chromatographic separation was achieved at an oven temperature of 140°C for 2 minute and injection port temperature of 280°C. The ramp was maintained at a temperature of 20°C/min to a final temperature of 290°C for 8 minute. Ions monitored were 245 being the primary ion, 189 secondary and 146 tertiary for fentanyl.

Results

Method Validation- Current analytical procedure is rapid, accurate and reproducible for simultaneous extraction and quantification of fentanyl cotinine in urine and plasma. Method was validated as per UNODC (United Nation Office of Drug and Crime) guidelines.

Linearity- Six point calibration curve from 0.62 to 40.0 ng/ml was constructed by plotting peak area versus concentration. Linear regression was ≥0.9899. This method would be able to quantitate fentanyl up to sub-nano levels.

Sensitivity- The limit of detection (LOD) and limit of quantization (LOQ) were 0.06ng/ml and 0.24 ng/ml (with one ml sample) and signal to noise (S/N) ratio was 3:1.

Precision- Intra and inter day precision was observed within range (Table-1). Matrix effect in urine and plasma was investigated using six lots of blank matrix form individual donors was ≤10% while test carry over was negligible.

Recovery- Mean recovery was around 85% in urine and 87%in plasma matrix.

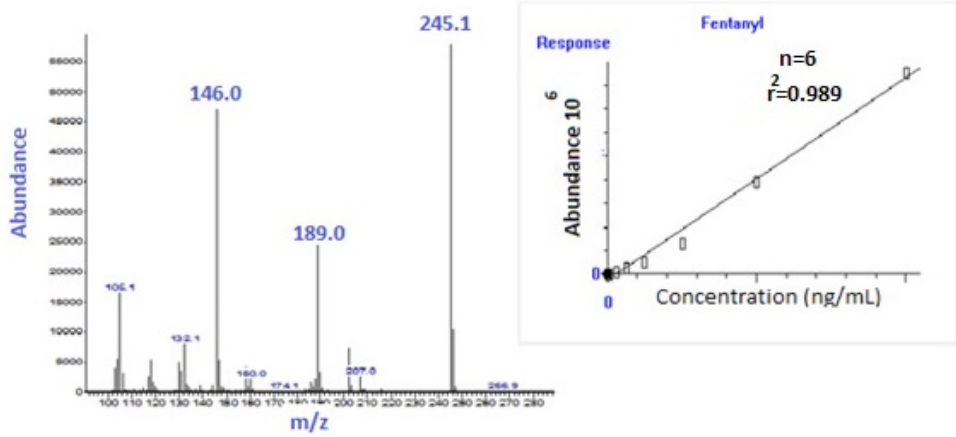

Fig.1 Mass Spectrum showing m/z along with Calibration curve for Fentanyl

Robustness- Robustness of the method was evaluated by introducing small changes in matrix, mobile phase and analyst. We report accuracy of 90% which is well within the range (80-120%).

Sample Stability- Since samples reconstitution was in ethyl acetate, we injected samples immediately to avoid solvent evaporation. Fentanyl in urine frozen at -80°C was found stable for 3 months while fentanyl in frozen plasma was stable for 6 months at -80°C.

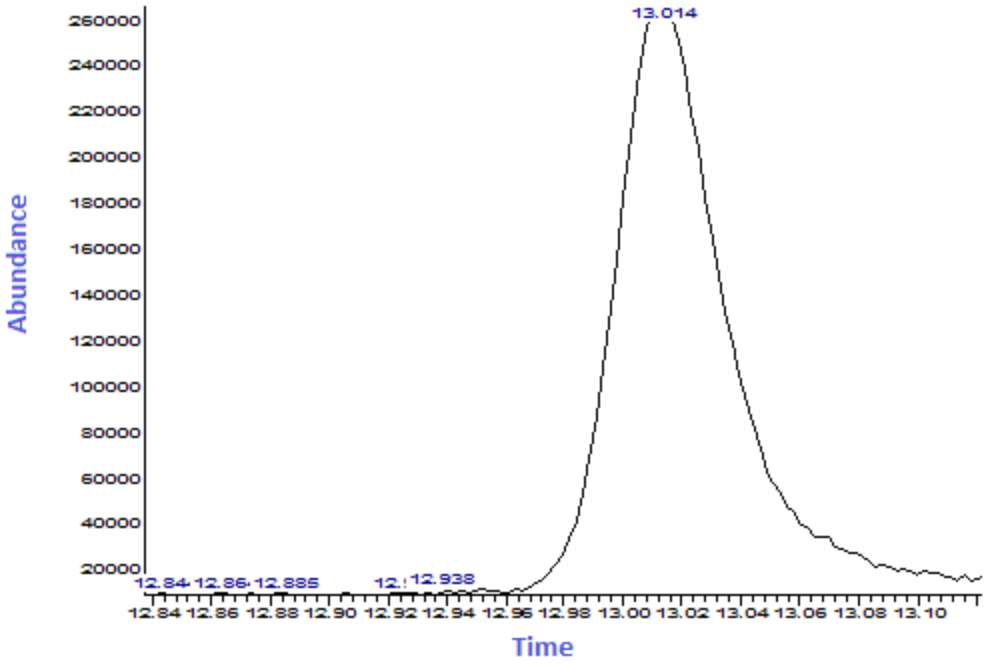

Fig.2 Chromatogram showing peak of Fentanyl

Table 1: Method validation parameters of Fentanyl standardization along with comparison other GC-MS technique.

Serum	**Our Results**	**Ahmad (2008)**	**Alphonse (2004)**	**Strano-Rossi (2010)**
Method	GC-MS	GC-MS	GC-MS	GC-MS
Linearity Confidence limit (ng/ml)	0.62-40	1-1500	0-933	0.5-50ng/ml
Linear Regression coefficient(r^2)	0.9899	0.99	NA	0.99
LOD (ng/mL)	0.06	3	3	0.08
LOQ (ng/mL)	0.24	4	3	0.5
Recovery (%)	>85%	70%	--	85%
Precision % Inter Day(1,5,40 ng/ml) Intra Day	 5.22% 1.5%			 9.88 5.7

Twenty four samples received were analyzed out of which 8 were of plasma while 16 were urine sample. Since for the screening of plasma samples cassettes are not available samples were quantified by GC-MS. Two samples were negative, while six had values ranged between 4.0 to 50.0ng/ml mean10.6±16.4. For blood or plasma cutoff is 2.0 ng/ml (immunoassay) .Urine samples were first screened by spot testing cassettes for which cutoff was 20.0ng/ml (Abon). Out of 16 urine sample nine were negative by cassette testing. Quantification by current analytical procedure matched the screening results. Negative samples had values between 0 -12 ng/ml. Nine on quantification using GC-MS there values lied between 0-12 ng/ml . Mean of 16 samples we analyzed was 25.36±29.34 (Graph-1).

Discussion

Numerous analytical techniques designed for detection and quantification of fentanyl via, High performance Liquid Chromatography (HPLC), HPLC-MS & GC as well as GC-MS have been reported till date. GC-MS technique is a unique and full proof tool for identification and quantification purpose [7]. Mass spectrometric detection provide electron ionization fragment of the molecules with mass to charge ratio, which determines the relative abundance of the ions by GC-MS without any doubt. For extraction of biological samples liquid as well as solid phase extraction techniques was applied. SPE has advantage over liquid-liquid extraction [8] as it is humble, rapid accurate with high degree of recovery (≥85%) clear extracts were obtained. This simple, sensitive & specific method can be functional for extraction and quantification of many samples at a time.

Few methods have been published previously for extraction of fentanyl (4) in different biological matrices. However SPE was carried out using bond elute cartridges offered good recoveries (Table-1). Fentanyl was screened in full mass scanning (SCAN) mode and compound identification was done with in-built National Institute of Standards and Technology research library (NIST). Quantification was done in Single Ion Monitoring (SIM) using GC-MS, the retention time (13.01 min) was determined by standard solution and no interference from co-extracted compound was seen nearby (fig1, 2). To prepare calibration curve and analyzing samples GC–MS was operated in SIM mode to enhance the sensitivity and selectivity. Six point calibration curve from 0.62 to 40.0 ng/ml was constructed by plotting peak area versus concentration. Linear regression was ≥0.9899. This method would be able to quantitate fentanyl up to sub-nano levels. The limit of detection and limit of quantification were 0.06ng/ml and 0.24 ng/ml with one ml sample and signal to noise (S/N) ratio was 3:1.

Furthermore, the current procedure was validated based on selectivity, linearity, accuracy, precision and recovery of the analytes (Table 1). Blank plasma samples were tested for endogenous interferences, and the anticipated fentanyl retention time regions were shown to be free from interference. The chromatographic conditions for the GC-MS method endow a well-defined separation of drug, without endogenous components. Typical chromatograms and spectrum are shown in Fig. 1, 2. The linearity ranged from 0.62 to 40.0 ng/ml and coefficient of determination was (r^2) >0.989. Within run precision and extraction recovery at 0.5 ng/ml were found to be 84.8%. At the lowest calibration point 0.1ng/ml signal to noise ratio was 3:1. This technique would prove beneficial for quantification of fentanyl in clinical and forensic samples. Further larger scale studies should be undertaken to better understand the implication of testing fentanyl and its metabolites in clinical population.

The described GC-MS method has the advantage of using one calibration curve for both low and high concentrations of fentanyl. Furthermore, solid phase extraction procedure is quick and has high degree of recovery of the analytes. The validation results indicate that the method is sensitive, specific, and reliable for the quantitation of high number of blood samples. In conclusion, we have described a method, for the fentanyl quantification in urine and plasma. Fentanyl recovery in synthetic and real urine samples was comparable. Results obtained by current analytical procedure were in agreement with screening by spot testing cassettes. The method was validated, showing good within-day and between-day precision, excellent accuracy and reasonable limits of quantitation 0.24ng/ml . As well, the stability of fentanyl in synthetic urine was also demonstrated over 12 weeks.

In conclusion summary a simple, selective, accurate and precise method for the analysis of fentanyl has been developed and validated. This procedure has good sensitivity and specificity along with good extraction efficiency even with smaller volume.

Conflict of Interest: The authors had no conflict of interest to declare in relation to this article.

Source of Funding: This work was supported by Centre for Addiction Medicine, NIMHANS Bangalore

Ethical Clearance- Ethical Clearance for was obtained from Institute Ethics committee

References

1. Kuhlman JJ Jr, MR, Valouch TJ, Behonick GS. Fentanyl use, misuse, and abuse: a summary of 23 postmortem cases. J Anal Toxicol. 2003; 27: 499-504.

2. Netaji A, MR, Ashraf H, Labaf A, Golshani K. Ketamine/propofol versus midazolam/fentanyl for procedural sedation and analgesia in the emergency department: a randomized, prospective, double-blind trial. Acad Emerg Med. 2011; 18: 800-6.

3. Poklis A. Backer R. Urine Concentrations of Fentanyl and Norfentanyl during application of Duragesic[R] Transdermal Patches. J Anal Toxicol. 2004; 28: 422-24.

4. Sabina Strano-Rossi, Álvarezm I, Tabernero M J, Cabarcos P, Fernández P, Bermejo AM et.al. Determinatikn of Fentanyl metabolite and analogs in Urine by GC/MS. J Appl Toxicol. 2010; 31: 649-54.

5. Ahmad H Malkawi, AMA-G, Peter A Crooks. Development of a GC-MS Assay for the Determination of Fentanyl Pharmacokinetics in Rabbit Plasma after Sublingual Spray Delivery. AAPS J. 2008; 10: 261-67.

6. Natasha Chandok, KDW. Pain Management in the Cirrhotic Patient: The Clinical Challenge. Mayo Clin Proc. 2010; 85: 451-58.

7. Huynh NH, Tyrefors N, Ekman L, Johansson M. Determination of Fentanyl in human plasma and fentanyl and norfentanyl in human urine using LC/MS/MS. J.Pharma.Biomed.Anal. 2005; 37: 1095-1100.

8. Silverstein JH, Rieders MF, McMullin M, Schulman S, Zahl K. An analysis of the duration of the fentanyl and its metabolites in urine and saliva. Anesth. Analg. 1993; 76: 618-21.