

## Gas Chromatography-Mass Spectrometric Analysis of Forensic Drug Flunitrazepam upon Exposure to UV Irradiation

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### Abstract

The environment may affect the forensic drug evidences in crime scene and is able to produce impurities, which contain vital information for tracing their origin of manufacture and can be used to provide link in crime scene investigation. In this work the response of forensic drug flunitrazepam to the UV irradiation was investigated by gas chromatography-mass spectrometry. We found the substantial change in GC pattern involving multiple GC peaks, indicating the complex reaction of degradation in flunitrazepam upon UV irradiation. GCMS analysis identified one of the GC components to be amino-flunitrazepam. The new GC peaks and the GC pattern change may serve as “chemical signatures” of flunitrazepam. Such information may promote the identification of the forensic drug flunitrazepam as a “chemical fingerprint” in forensic sciences.

**Keywords:** Flunitrazepam; Gas chromatography; Mass spectrometry; Impurity; UV irradiation; Environment

### Introduction

Flunitrazepam is one type of benzodiazepines, which are controlled substances and belongs to date-rape drugs [1-8]. The chemical structure of flunitrazepam is shown in Figure 1, which has a chemical formula of  $C_{16}H_{12}FN_3O_3$ . Flunitrazepam is odorless, however it is available the pills and tablets are produce from a white (often yellowish) powder. It is used to relieve anxiety, insomnia, muscle spasms and epileptic seizures. Although it is essential in treating medical conditions, flunitrazepam is one of the misused drugs on the market. Flunitrazepam shares with other benzodiazepines the risk of abuse, misuse, and sense of dependence. Some people use flunitrazepam (Rohypnol) to sleep, relax, and to create a euphoric feeling. Flunitrazepam has several major side effects like drowsiness, dizziness, slurred speech, breathing problems, and rapid heartbeat. The benzodiazepines have been available through the internet and become one of the serious issues in forensic cases [8-11].

A sensitive a rapid method using gas chromatography with mass spectrometry was developed for simultaneous determination of the benzodiazepines in blood [12-14]. Using liquid-liquid extraction, gas chromatography with tandem mass spectrometry is used successfully to detection of 136 analytes of drugs including benzodiazepines, antidepressants, neuropeptides, beta-blockers, antidiabetics, in human blood plasma. Liquid chromatography with mass spectrometry has been used in simultaneous analysis of benzodiazepines and metabolites in hair samples [15-21]. Six benzodiazepines, flunitrazepam, alprazolam, lorazepam, lormetazepam, diazepam, and tetrazepam, in vitreous humor were analyzed by high performance liquid chromatography

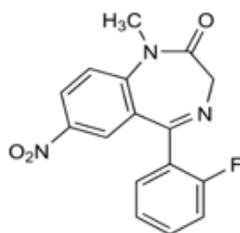


Figure 1: Chemical structure of flunitrazepam.

with a photodiode array detector using solid-phase extraction with the limit detection of 3 ng/mL [22].

Forensic drugs may generate impurities during their manufacture, storage, and exposure to environment. In particular, the crime scenes may include varies of environment, which may affect the illicit drugs and are able to produce impurities. These impurities cause the “forensic drug impurity signature” using a number of bioanalytical methodology and vital information for tracing their origin of manufacture and time of the incident, and can be used to provide link in crime scene investigation [23,24]. In this work the responses of flunitrazepam to the UV irradiation were investigated by gas chromatography-mass spectrometry. We observed the substantial change in GC pattern involving multiple GC peaks under UV irradiation conditions. One of the GC components was identified to be amino-flunitrazepam. The experimental data may be valuable in promoting the identification of the forensic drug flunitrazepam as a “chemical fingerprint” in forensic sciences.

### Materials and Methods

#### Chemicals

Flunitrazepam (1 mg/mL in methanol) was purchased from Cerilliant and used for prepared working solution (0.10, 0.050, and 0.025 mg/mL in methanol) for GCMS analysis. Methanol (HPLC grade, 99.9%) was purchased from Acros and used without further purification.

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## UV irradiation treatment of flunitrazepam

The UV irradiation was proved by an UV light source (Blak-Ray B-100AP High Intensity UV Lamp, 100 W, 365 nm). The flunitrazepam methanol solutions were exposed under UV light for 0, 10, 20, 30, 40, 50, and 60 min. The distance between the UV light and flunitrazepam sample were 20 cm to minimize the effect of possible elevated temperature generate by UV lamp. The control experiment using the methanol solution under the identical condition were conducted and examined by GCMS for potential contamination and interference.

## GCMS analysis

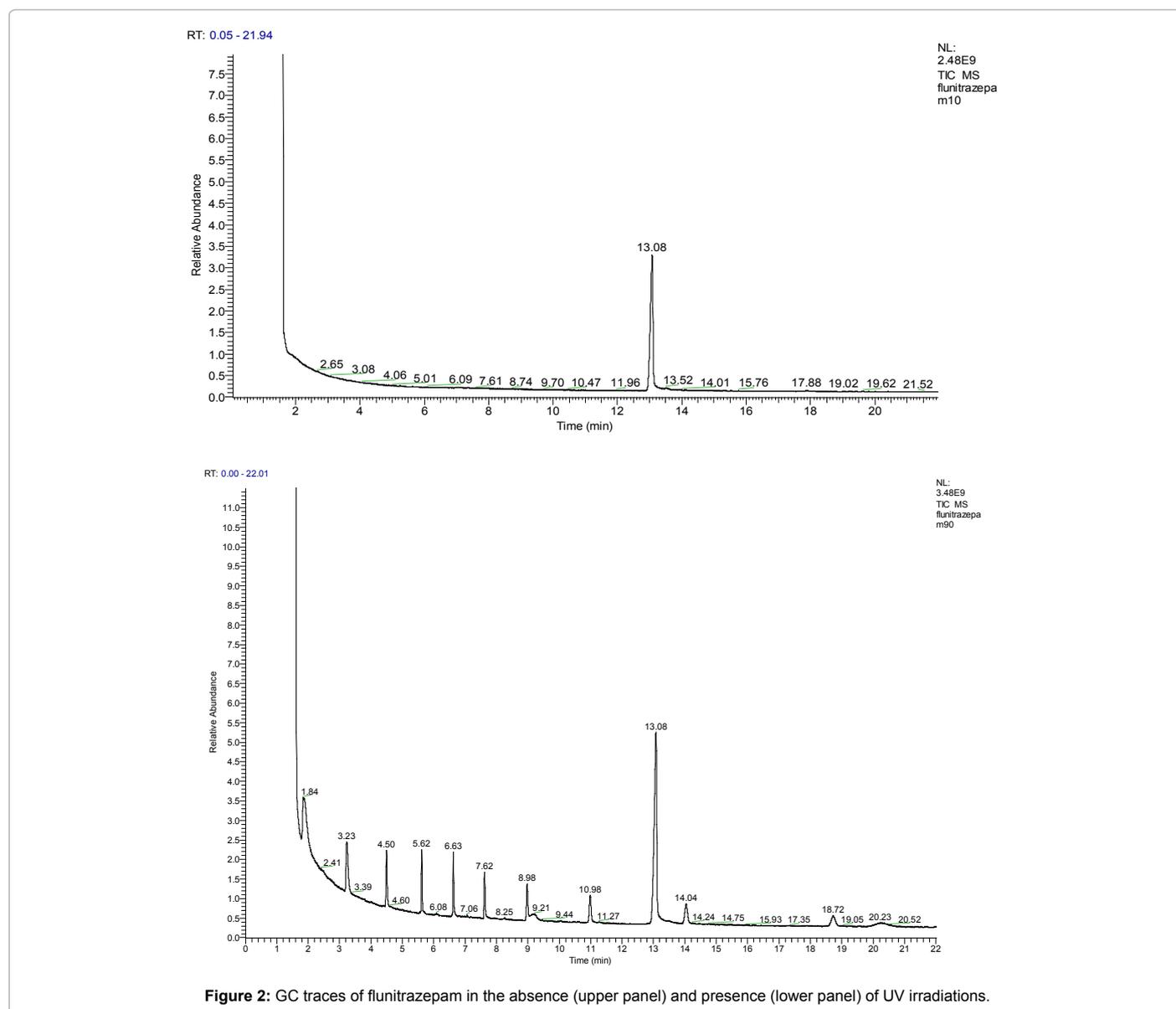
The flunitrazepam samples (1-3  $\mu$ L) before and after UV treatment were analyzed by a GCMS system (Thermal Fisher), including the TRACE GC Ultra 2.2 gas chromatography, ISQ 1.0 SP4 mass spectrometer, and TRIPLUS RSH 1.1 auto sampler. A capillary GC column (TR 5.7 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m) was used for chromatographic separation. The column temperature is programmed by holding at

150°C for 2 min, then increasing to 225°C at a rate of 15°C per min, and finally keeping at 225°C for 15 min. The injector temperature with split less mode was set at 250°C. The carrier gas was the ultra high purity helium gas (Airgas) at a flow rate of 1 mL/min. Data analysis was conducted using Xcalibur 2.2 software (Thermal Fisher).

## Results and Discussion

### Effect of UV irradiation on flunitrazepam

Figure 2 (upper panel) showed the typical gas chromatograph of flunitrazepam in methanol solution before and after UV light treatment. In the absence of UV irradiation, a single GC peak at 13.1 min was observed. Upon UV light treatment, more than ten GC peaks were observed (Figure 2, lower panel), suggesting the photosensitivity of flunitrazepam to UV irradiation. The eight GC peaks at 1.84, 3.23, 4.50, 5.62, 6.63, 7.62, 8.98, and 11.0 min showed the short retention time than flunitrazepam, suggesting that the UV irradiation causes the multiple lower boiling point components than flunitrazepam. We also



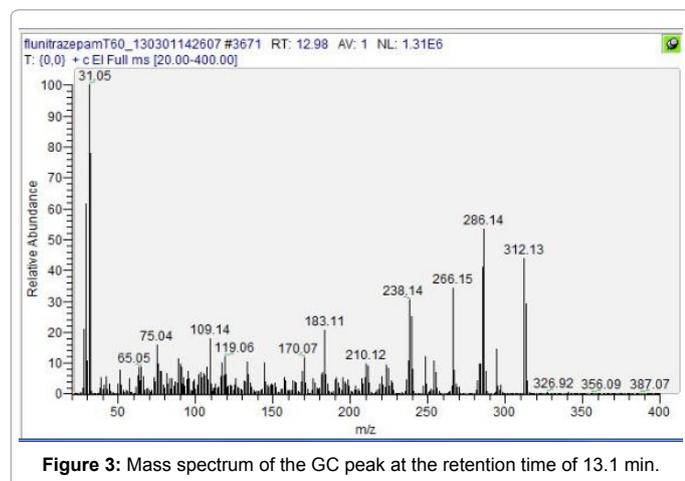


Figure 3: Mass spectrum of the GC peak at the retention time of 13.1 min.

found the three likely higher boiling point components at 14.0, 18.7, and 20.2 min.

Figure 3 showed the mass spectrum of the GC peak at 13.1 min, which showed major signals at the mass to charge ratio ( $m/z$ ) 65, 75, 109, 119, 170, 183, 210, 238, 266, 286, 312, and 313. The GC peak at 13.1 min was identified to be flunitrazepam by the mass data library using Xcalibur 2.2 software (Figure 4). The typical mass spectral features of flunitrazepam are 238, 266, 285, 312, and 313 [25]. The  $m/z$  313 was assigned to the molecular ion peak of flunitrazepam. The  $m/z$  285 peak is likely due to the loss of CO ( $313-28=285$ ), and the  $m/z$  266 peak may be produced by removing of H and  $\text{NO}_2$  ( $313-46-1=266$ ). The  $m/z$  238 peak may be assigned to loss of  $\text{HNO}_2\text{CO}$  fragment ( $313-75=238$ ).

### Reaction mechanism of UV-induced photo degradation of flunitrazepam

As shown in Figure 2 (lower panel), UV irradiation cause significant change in GC profiles of flunitrazepam with the appearance of more than ten new GC peaks. However, the identification of the majority unknown GC peaks was not successful using the mass spectral database. It indicated that the reaction of photodegradation of flunitrazepam under UV irradiation conditions is complex. The complex reaction under strong light intensity was observed in the case in the photodamage of pheophytin in the photosystem II reaction center from spinach and reaction center from *Rb. sphaeroides* [26,27]. The multiple products of pheophytin photodamage were found using the high performance liquid chromatography (HPLC).

To investigate the reaction mechanism of the UV-induced photodegradation of flunitrazepam, the GC analysis of flunitrazepam over the 60 min period were conducted. The GC profiles of flunitrazepam exposed to UV irradiation over the retention time of 11-15 min revealed a gradually increase of two new GC peaks at 13.5 and 14.1 min in the chromatograph of flunitrazepam (data not shown). The GC peak at 14.1 min is the same as that in Figure 2. The additional GC peak at 13.5 min was increased over time, suggesting that it is one of the photodegradation products of flunitrazepam by UV irradiation. The identification analysis using the mass spectral database implied that the GC peak at 13.5 min is amino-flunitrazepam (Figure 5). The nitro group in flunitrazepam is likely converted into amine group by UV irradiation.

In conclusion, flunitrazepam is a benzodiazepine drug that can be used for medical as well as recreational purposes. The analysis of

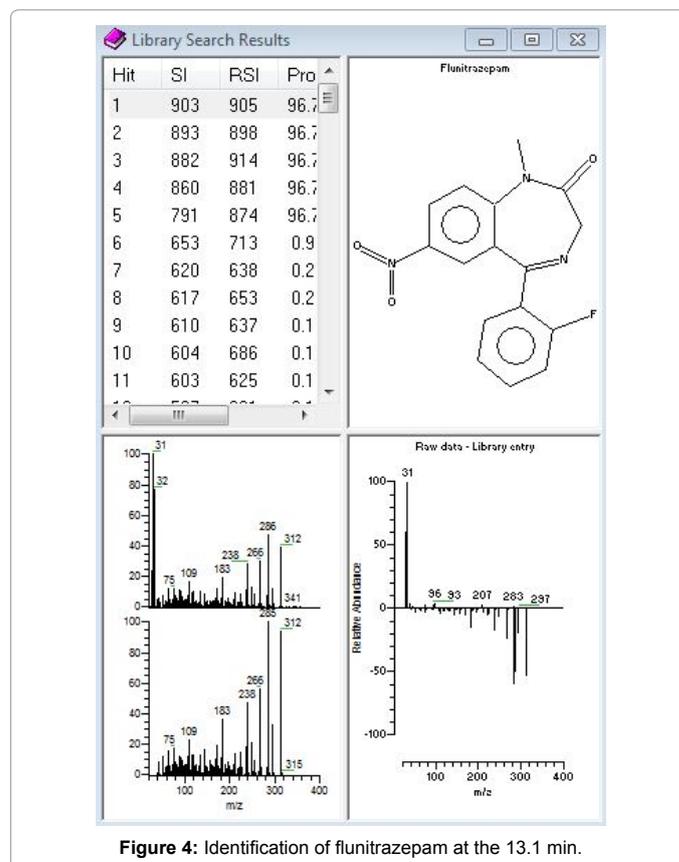


Figure 4: Identification of flunitrazepam at the 13.1 min.

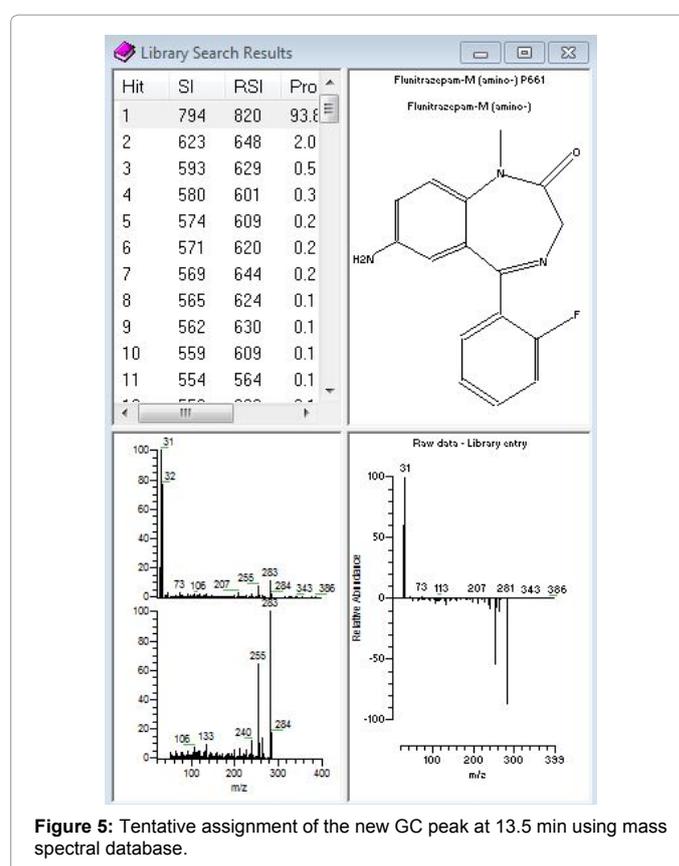


Figure 5: Tentative assignment of the new GC peak at 13.5 min using mass spectral database.

this drug was conducted by gas chromatography mass spectroscopy to explore the environmental effect (UV irradiation) on flunitrazepam. We observed more than ten new GC components of photodegradation reaction in flunitrazepam by GCMS in the presence of UV irradiation. Further work on the kinetics of the photoreaction of flunitrazepam is worthwhile. The experimental procedures of human hair DNA samples were established. One new GC peak at 13.5 min was identified to be amino-flunitrazepam and seems due to the reduction of nitro group. Further work on the kinetics of the photoreaction of flunitrazepam is worthwhile. We noticed the substantial change in GC pattern involving multiple GC peaks in the retention time of 2-13 min. The new GC peaks and the GC pattern change may serve as “chemical signatures” of controlled substances. In crime scene the forensic drugs including flunitrazepam are often found. These substances may be exposed to the environment with elevated temperature or strong sun light for prolong period of time. The experimental data presented in this work may provide useful information regarding the potential link between the forensic drug evidences and suspects. In addition, such information may provide novel insights into the identification of the illicit drugs as a “chemical fingerprint” or construction of “illicit drug signature” database in forensic sciences.

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