Identification of Furanoylfentanyl and its Metabolites in Human Urine

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Abstract

HPLC-HRMS method for detection of Furanoylfentanyl and its metabolites in the urine is developed. For this it is proposed the implementation of an approach using targeted searches for finding the most probable metabolites, basing on their calculated exact mass and fragmentation regularities. HRMS spectrum of Furanoylfentanyl and its metabolites are obtained.

Keywords: Designer drugs; Fentanyl structural analogues; HPLC-MS/MS; High-resolution mass spectrometry

Introduction

In the last years new synthetic drugs have become widespread, affecting a variety of brain receptors. Derivatives and fentanyl analogues are a class of high-performance synthetic narcotic analgesics acting primarily on the µ-opioid receptors. 200 fentanyl analogues have been synthesized now, which differ in the efficiency and duration of action. The fentanyl derivatives are often used as substitutes of heroin in an illegal transfer. However, because of the ultra-high analgesic activity and non-permanent composition of mixes in street drugs, taking fentanyl derivatives often leads to fatal overdose [1].

Furanoylfentanyl \(N\)-(1-(2-phenylethyl)-4-piperidinyl)-N-phenylfuran-2-carboxamide, "Fu-F") is a fentanyl derivative, which was synthesized and patented in 1986, and has a furanyl ring in place of the methyl group, adjacent to the carboxyl bridge (Figure 1). Furanoylfentanyl has an ED-50 in mice of 0.02 mg/kg. Furanoylfentanyl is illegal in Sweden as of January 2016 [2,3].

Some analogs of fentanyl can be identified in the screening urine by GC/MS. However, the concentration of most illegal analogues and its metabolites in the biological fluids is too small to ensure their detection by this method. Furanoylfentanyl rarely detects in the ordinary screening of biological fluids by GC/MS.

The purpose of this thesis is the identification of Furanoylfentanyl and its metabolites in urine using HPLC with high-resolution mass spectrometry (HPLCHRMS). As shown previously [1-4], Furanoylfentanyl and a number of other structural fentanyl analogues is one of new synthetic drugs, and its use often leads to fatalities.

Previously a number of new synthetic drugs and its metabolites were described [5-8]. The development of new methods of non-targeted screening for toxicological purposes is still relevant.

Figure 1: Structure of furanoylfentanyl.

Experimental

Selecting test object. For the test object, we chose the urine of a 28-year-old man suspected in using narcotic substances.

Sample preparation

HPLC–MS/MS analysis is performed using dilute and shoot procedure. For this, 150 µl of acetate buffer with pH=9 was added to 1 ml of the explored urine sample and centrifuged at 12000 rpm for 5 min. After that, 30 µl of the sample was injected into a chromatography-mass spectrometry system equipped with an on-line SPE system.

Chromatography-spectrometry analysis

An Agilent 1260 UPLC system coupled with a tandem quadrupole (Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS detector; Agilent Technologies) were used. A Zorbax Extend-C18 RRHT 2.1 mm × 50 mm × 1.8 µm column (Agilent Technologies) was used for separation of Fu-F and its metabolites. The column was stored at 45°C. The mobile phase consists of 0.1% aqueous formic acid (A) and acetonitrile (B). The following gradient program was used: 0-2.5 min 5% B, linear gradient elution from 5% B to 99% B for 2.3 min, plateau at 99% B to 15 min, back to 5% B at 1 min and equilibration for 6 min. The flow rate was 0.3 mL/min. The QTOF instrument was operated with an ESI ion source in positive ion under the following conditions: ion source temperature, 350°C; capillary voltage, 3.5 kV; fragmentor voltage, 120 V; skimmer voltage, 65 V; sheath and aux gas flow rate were set at 8 L/min; precursor ion scanning range: 100–500 Da; product ion scanning range: 50–500 Da. Nitrogen was used as a collision gas, the collision gas pressure in collision cell was 1.37 kPa, and the collision energy was set at 20 eV. The analysis was conducted in the Auto-MS mode with the automatic selection of the most intense five precursor ions and their subsequent fragmentation in collision cell. In the selection of the precursor ion, the specified transmission window of quadrupole was 1.3 Da. Adjustment, operation of the instrument and data processing was under MassHunter Workstation Software B.05.00 control. Preliminary treatment by on-line...
SPE method was performed in a PLPR-S cartridge (2.1×12.5 mm, part No Agilent 5982-1271) representing a styrene-divinylbenzene-based polymer sorbent. After injecting 50 µl of the sample, the cartridge was purged with 2.5 ml of 5% ethanol water solution with pH=9 in order to wash off ballast substances, then working eluent was pumped with the cartridge was with subsequent elution of analytes directly into the column. The ultra-pure grade water (Agilent), acetonitrile for HPLC–MS (Panreac), and 85% formic acid (Panreac) were used.

Results and Discussion

Determination of concentration psychoactive substances using commercially available standard is not the main purpose of this article. Such studies are currently already undertaken by other authors [9,10]. Moreover, there are legal difficulties related to the acquisition of such standards in our country. The primary objective of this study is to show methods of identifying (or predicting) a substance and its main metabolites using HRMS.

As showed before [4], HPLC–HRMS is a powerful tool for searching metabolites of poorly studied xenobiotics. In this thesis, we used this method and the previously described approaches in searching both for the main substance and its metabolites.

Studying of the chromatogram object obtained under the above mentioned conditions by HPLC–MS/MS method was started by searching for the native compound furanoylfentanyl, because it may be excreted from the body in unchanged form. Since protonated molecular ions of this compound have the accurate mass of 375.2074. We used the computer software to create a chromatogram based on the specified 375.2074 Da ion with a 10 ppm search window (Figure 2). Besides, the spectrum of ion products was obtained for this compound with collision energy of 20 Ev (Figure 3).

Figure 2: Chromatogram of urine sample containing furanoylfentanyl, based on a 375.2074 Da ion.

Figure 3: MS/MS spectra obtained as a result of dissociation during the collision of 375.2074 ion.
As we can see, the main fragment ions for this compound have accurate masses m/z: 188.1448, 105.0702, 134.0955, 254.1179. The formation of these ions can be explained by the fragmentation, presented in Figure 4.

In addition, the resulting spectrum as seen has common ions with the CID spectrum of fentanyl, presented in the library Broecker, Herre, and Pragt master forensic and toxicology accurate mass compound database with accurate mass MS/MS spectra (Figure 5). General fragmentation patterns are the result of a similar chemical structure of the compounds.

A product of monohydroxylation of the initial compound was suggested as one of probable metabolites. Its calculated mass is 391.2016 Da (the mass of a protonated molecular ion). During the study of the ion’s chromatogram with 10 ppm search window, the following chromatogram was obtained. However, monohydroxy metabolite of this compound has not been detected.

Also, known as the major metabolite of fentanyl is norfentanyl. For the present compound by appropriate metabolite must be a compound N-phenyl-N- (piperidin-4-yl) furan-2-carboxamide with the payment of the molecular ion mass of 271.1441. However, the compound with this molecular weight is also absent in the studied sample.
Next was applied other search algorithm to find metabolites of furanoylfentanyl. This algorithm is based on the assumption that fragmentation of metabolites will be similar to the native compound. Thus, the most intense fragment ions characteristic for the metabolites is m/z 188.1439, as well as for native compound. In the obtained chromatogram we found a few of m/z ms spectra comprising ion m/z 188.1439. Based on the obtained mass spectra and fragmentation of laws, for each of the spectrums have been proposed metabolites structure. Mass spectra and proposed metabolites structures are shown at Figure 6. Chromatograms for selected molecular ions ([M+H]+) characteristic of these metabolites are shown in Figure 7. The order of elution is the same sequence with the compounds at Figure 6A-6E. The last peak is native furanoylfentanyl.

The next step was to find metabolites that contain more oxygen atoms. In the obtained chromatogram, we searched the spectra containing fragment ion 204.1388, which is formed by introducing oxygen into the structure described previously ion 188.1439. In the obtained chromatogram we found a few of ms / ms spectra comprising ion m/z 204.1388. Based on the obtained mass spectra and fragmentation patterns, for each of the spectrums have been proposed metabolites structure. Mass spectra and proposed metabolites structures are shown at Figure 8. Chromatograms for selected ions characteristic of these metabolites are shown in Figure 9. The order of elution is the same sequence with the compounds at Figure 8. The last peak is native furanoylfentanyl.

Conclusion

Thus, in the course of this thesis the possibility of using HPLC-HRMS method have been demonstrated as the primary and confirmatory methods analysing of urine human sample, who took a synthetic opioid Furanoylfentanyl, as an example. In addition to the basic substance in the urine have been found its metabolites and as a result its structure are proposed. Moreover, it is considered the basic way of identification by HPLC-HRMS using prediction accurate weight formed metabolites in aggregate with the study of the fragmentation protonated molecular ions in the collision cell and comparing this fragmentation with established spectrum native substances.
Figure 9: Chromatogram of urine sample for selected molecular ions (MH+) characteristic of some metabolites.

References

1. Mohr AL, Friscia M, Papsun D, Kacinko SL, Buzby D, et al. (2016) Analysis of Novel Synthetic Opioids U-47700, U-50488 and Furanyl Fentanyl by LC–MS/MS in Postmortem Casework. Journal of Analytical Toxicology 40: 709-717.

2. Helander A, Bäckberg M, Beck O (2016) Intoxications involving the fentanyl analogs acetyl-fentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clinical Toxicology 54: 324-332.

3. Feasel MG, Wohlfarth A, Nilles JM, Pang S, Kristovich RL, et al. (2016) Metabolism of Carfentanil, an Ultra-Potent Opioid, in Human Liver Microsomes and Human Hepatocytes by High-Resolution Mass Spectrometry. The AAPS Journal 18: 1489-1499.

4. Labutin AV, Temerdashev AZ (2015) Nontarget screening of the markers of synthetic cannabinoids in urine using HPLC–MS/MS. Journal of analytical chemistry 70: 1620-1628.

5. Grigoryev A, Kavanagh P, Melnik A (2012) The detection of the urinary metabolites of 3-[(adamantan-1-yl) carbonyl]-1-pentylinole (AB-001), a novel cannabimimetic, by gas chromatography-mass spectrometry. Drug testing and analysis 4: 519-524.

6. Temerdashev AZ, Grigor’ev IM, Ryal’chenko IV (2014) Evolution of new narcotic substances and methods of their determination. Journal of Analytical Chemistry 69: 817-844.

7. Sobolevsky T, Prasolov I, Rodchenkov G (2012) Detection of urinary metabolites of AM-2201 and UR-144, two novel synthetic cannabinoids. Drug testing and analysis 4: 745-753.

8. Sobolevskii TG, Prasolov IS, Rodchenkov GM (2010) Mass-Spektrometriya 7: 175-182.

9. Guarrieri D, Rapp E, Roman M, Druid H, Kronstrand R (2017) Postmortem and Toxicological Findings in a Series of Furanylfentanyl-Related Deaths. Journal of analytical toxicology 41: 242-249.

10. Helander A, Bäckberg M, Beck O (2016) Intoxications involving the fentanyl analogs acetyl-fentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clinical Toxicology 54: 324-332.