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Seizure of illicitly produced *para*-fluorofentanyl: quantitative analysis of the content of capsules and tablets

D. de Boer [a,b,*], W.-P.J. Goemans [b], V.R. Ghezavat [b], R.D. van Ooijen [b], R.A.A. Maes [b]

[a] *Instituto Nacional do Desporto, Laboratório de Análises de Dopagem e Bioquímica, Av. Prof. Egas Moniz (Estádio Universitário), 1600-190 Lisbon, Portugal*

[b] *Department of Human Toxicology, Utrecht Institute of Pharmaceutical Sciences (UIPS), Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands*

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Abstract

A gas chromatography/mass spectrometry (GC/MS) method for the quantification of *para*-fluorofentanyl (pFF) in powder and powdered samples was developed and validated. The method was applied on a seizure of capsules and tablets, that had been confiscated at an illicit production site in the Netherlands. The investigated capsules and tablets contained pFF in the range of 33.8–408.7 μg. As caffeine was detected as being an adulterant, a HPLC/UV method for the quantification of caffeine in capsules and tablets was also validated and applied. Caffeine was detected in the range of 25.6–108 mg per capsule or tablet. Based on an extrapolation of pharmacological and toxicological data of fentanyl, it can be argued that the highest detected single dose of pFF could be lethal, when administered orally. However, the large variability of the doses observed for pFF could mislead abusers, potentially leading to multiple doses and thus overdosing.

Keywords: Forensic toxicology; *para*-Fluorofentanyl; Caffeine; Illicit preparations; Gas chromatography/mass spectrometry

1. Introduction

The group of fentanyl drugs can be classified into registered therapeutic fentanyls and illicitly produced fentanyl and analogues [1]. From a toxicological point of view, both sub-groups have their typical characteristics. For example, the abuse as well as intoxications of registered therapeutic fentanyls is worldwide a frequently occurring phenomenon. It is mainly related to drug addicts, health care and forensic professionals, accidental mistakes in medications and intentional overdoses in suicides [2–11].

On the other hand, intoxications with illicit fentanyl and/or one of its analogues take place less frequently and may occur occasionally with drug addicts, when a batch of such compounds is released in the street-scene [12–17]. Until 1994, the

* Corresponding author. Tel.: +351-21-795-4000; fax: +351-21-797-7529.
E-mail address: douwedeboer@mail.telepac.pt (D. de Boer).

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issue of illicit fentanyl analogues was restricted to the United States [1], but since then, also Western Europe has been faced with that problem [17–20].

This paper describes the quantitative analysis of the contents of capsules and tablets containing the illicit fentanyl analogue *para*-fluorofentanyl (pFF; Fig. 1). The formulations had been confiscated at a certain illicit production site in the Netherlands. A related report of the case history has been described elsewhere [21].

2. Materials and methods

2.1. Materials

Phenacetine and caffeine were gifts of the Netherlands Institute of Drugs and Doping Research (Utrecht, The Netherlands). pFF was a generous gift of Janssen Pharmaceutica (Turnhout, Belgium). Fentanyl citrate was purchased from Diosynth BV (Oss, The Netherlands).

2.2. Initial preparation of capsules and tablets

Prior to analysis, the confiscated samples and their content were weighed. Samples were grinded, in case of tablets, or opened, in case of capsules. Twenty five mg of the homogenised sample were dispersed in 5 mL of demineralised water (= working solution for pFF). An aliquot of the obtained dispersion was diluted 1250 times (100 μl in sufficient volume to produce 125 ml) with demineralised water (= working solution for caffeine).

2.3. Isolation of pFF

Twenty micrograms of the internal standard fentanyl, 200 μl of a 25% potassium hydroxide solution (w/v), and 200–250 mg of sodium sulphate were added to 2 ml of working solution for pFF or calibrator solutions (calibrator solutions ranging from 0.30 to 30.0 μg/ml of pFF). The compounds of interest were extracted twice with 2 ml of diethyl ether. The organic phases were combined and transferred to point shaped tubes and the excess of organic phase was evaporated under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 200 μl of methanol (= final solution for pFF).

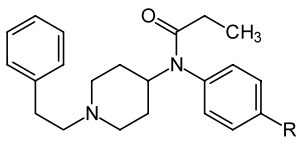

Fig. 1. Chemical structural formulas of fentanyl (R = H) and *para*-fluorofentanyl (R = F).

2.4. GC/MS analysis of pFF

GC/MS analysis was performed with a HP GC 5890 series II (Hewlett Packard, Waldbronn, Germany) coupled to a HP 5972 mass selective detector (Hewlett Packard). The GC/MS apparatus was equipped with CP-Sil 8 CB low bleed/MS column, length 30 m, inner diameter 0.25 mm and film thickness 0.25 μm (Chrompack, Bergen op Zoom, The Netherlands) and HP 6890 series automatic injector (Hewlett Packard). The temperature during an analysis run was maintained at 100 °C for 1 min, ramped to 290 °C at 20 °/min and maintained there for 4 min. The temperatures of the injection port and transfer line were 280 and 300 °C, respectively. Helium was used as a carrier gas at a flow rate of 0.8 ml/min. The pressure was kept constant using the electronic pressure control mode. Tuning was performed according to the manufacturer's recommendations. A sample volume of 2 μl of the final solution was injected in the split mode (1:50). Mass spectrometric analysis was performed in the electron ionisation mode at the standard 70 eV in the selected ion monitoring mode. The selected ions were for pFF at *m*/*z* 164, 207 and 263 and for fentanyl at *m*/*z* 146, 189 and 245, respectively [22].

2.5. Isolation of caffeine

Fifty micrograms of the internal standard phenacetine and 200–250 mg of a mixture of sodium hydrogen carbonate and sodium carbonate (2:1, w/w) were added to 2 ml of working solution for caffeine or calibrator solutions (calibrator solutions ranging from 0.72 to 24.0 μg/ml of caffeine).

The compounds of interest were extracted with 5 ml of a mixture of chloroform and isopropanol (85:15, v/v). The organic phase was transferred to point shaped tubes and excess of organic phase was evaporated under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 1 ml of methanol (= final solution for caffeine).

2.6. HPLC/UV analysis of caffeine

HPLC/UV analysis was performed with an AB 510 solvent delivery system (Applied Biosystems Nederland, Nieuwerkerk a/d IJssel, The Netherlands) coupled to AB Spectroflow 783 programmable UV detector (Applied Biosystems). The system was equipped with a PE ISS-101 autosampler (PerkinElmer Benelux, Oosterhout, The Netherlands) and a PE LCI-100 integrator (PerkinElmer). A RCSS Guard-Pak™ precolumn C18 (Waters Chromatography BV, Bergen op Zoom, The Netherlands) was used as a guard column for the analytical column, a cartridge (800 × 10 mm) packed with Nova-Pak® C18 spherical material 4 μm (Waters Chromatography BV) in combination with a RCM compressing module (Waters Chromatography BV). The mobile phase consisted of demineralised water and methanol (65:35, v/v) at a flow rate of 1.3 ml/min and was applied isocratic at ambient temperature. A sample volume of 20 μl of the final solution was injected. Caffeine and phenacetine were detected at 273 nm.

3. Results

The validation parameters, that were considered appropriate (Table 1), were selected based on the assumption that the methods would be applied during a short interval and exclusively for those pharmaceutical formulations that had been confiscated at that time. Accordingly, parameters that express the quality during a short period were relevant. Selection of validation parameters is a typical dilemma for forensic and toxicological laboratories in general, as those laboratories are dealing sometimes with analytical methods that are used very sporadically. The results of the validation parameters of the applied methods are summarised in Table 1. The initial goal was to use a HPLC/UV method for the quantification of pFF [23], but this kind of methodology proved not to be selective enough to measure pFF in presence of relatively high amounts of caffeine. Therefore, a GC/MS method was evaluated and approved. No interferences were observed for the chosen methods. The linear regression curves of pFF presented relatively a high intercept, because the curve did not show a linear behaviour below a concentration of 1 g/ml.

The results of the analysis of the confiscated samples are shown in Table 2. Samples subclassified with A, B and C are from the same batch of samples. Visual inspection of the capsules and tablets indicated that the formulations had similar appearances from out-side. The following observations were made: (1) in respect to the tablets it was noted that the quality in terms of hardness was insufficient. Handling resulted in crumbling and breaking. (2) Of some capsules within the same batch, the contents had not always a uniform colour.

The formulations contained pFF in the range of 33.8–408.7 μg. The wide range was due to variability in the total weight of the formulations within and between the batches and to the variability in weight percentage of pFF between the batches (0.14–1.57‰). Caffeine was detected in the range of 25.6–108 mg. The weight percentage of caffeine in the formulations was relatively constant (27.7–30.2% for capsules and 36.9–41.5% for tablets).

Also at hand were powders used at the illicit production site to prepare the respective formulations. The composition of these powders corresponded either to that of the analysed formulations or to bulk powders used to make the formulations (data not shown in Table 2).

4. Discussion

Compared with a GC/MS method for fentanyl [24], the limit of detection (LOD) of the GC/MS analysis of pFF for this method is in the same range, namely 0.4 versus 0.15 μg/ml. For the

Table 1
Analytical validation parameters of applied methods

Parameter[a]	Caffeine[b]	pFF[b]
Recovery		
Q_l	88.2%	97.8%
Q_h	93.2%	100.1%
Accuracy		
Q_l	99.2%	138.0%
Q_m	103.5%	104.0%
Q_h	98.8%	100.7%
Repeatability[c]		
Q_l	1.19 ± 0.02 (1.94%; $n = 5$)	1.43 ± 0.02 (1.60%; $n = 3$)
Q_m	6.21 ± 0.08 (1.31%; $n = 5$)	10.13 ± 0.62 (6.07%; $n = 3$)
Q_h	17.8 ± 0.22 (1.24%; $n = 5$)	17.9 ± 1.65 (9.20%; $n = 3$)
Reproducibility[c]		
Q_l	1.23 ± 0.09 (7.16%; $n = 6$)	1.38 ± 0.25 (17.90%; $n = 5$)
Q_m	5.98 ± 0.15 (2.44%; $n = 6$)	10.4 ± 0.56 (5.34%; $n = 5$)
Q_h	17.8 ± 0.49 (2.74%; $n = 6$)	20.1 ± 0.89 (4.42%; $n = 5$)
Stability[c,d]		
Q_m	Not determined	9.55 ± 0.09 (0.94%; $n = 5$)
LOD	0.25	0.15
LOQ	0.6	7
Linearity	0.7–24	1–30
Correlation of coefficient[c]	0.99971 ± 0.00008 (0.01%; $n = 7$)	0.999 ($n = 1$)
Slope[c]	0.0035 ± 0.0250 (714%; $n = 7$)	0.2136 ($n = 1$)
Intercept[c]	0.2034 ± 0.0077 (3.79%; $n = 7$)	0.2142 ($n = 1$)

[a] Q's are quality control samples; for caffeine $Q_l = 1.2$ μg/ml, $Q_m = 6$ μg/ml, $Q_h = 18$ μg/ml and for pFF $Q_l = 1$ μg/ml, $Q_m = 10$ μg/ml, $Q_h = 20$ μg/ml; LOD, limit of detection; LOQ, limit of quantification.
[b] Absolute concentrations are given as μg/ml.
[c] Values are expressed as mean ± S.D. (CV).
[d] Stability was determined after sample preparation and storage at room temperature during 24 h.

purpose of this study, this was more than sufficient. The limit of quantification (LOQ) for pFF was relatively high, namely 7 μg/ml. The reason was probably that near the LOD the current method had an accuracy and repeatability which were not satisfactory. Above the LOQ the method is nevertheless more than adequate and the values found for pFF are in very good agreement with initial reported values based on units of fentanyl equivalents as reported elsewhere [21].

The scientific knowledge regarding pFF is limited to the analytical detection possibilities of the parent compound [25–28], the description of aspects of the illicit synthesis processes [19], some structure–activity studies [28,29] and some information about opioid receptor selectivity [30]. Although, only a few structure–activity studies are available, it has been suggested that pFF has a similar pharmacological potency as fentanyl [29,31]. Unfortunately, no specific information concerning the toxicology of pFF is available [31]. However, its abuse is considered not to be without serious risks. Most of the detailed toxicological information could be extrapolated from fentanyl itself. Serious side effects of fentanyl may include profound respiratory depression, bradycardia, hypotension, chest wall rigidity, nausea, vomiting and convulsions [32]. Therefore, after intravenous administration of doses of fentanyl > 200 μg artificial respiration should be applied [33]. In contrast to this, it should be noted that oral administrations of fentanyl ranging from doses of 500–5000 μg did not result into intoxication [19,32,34]. Apparently, limited intestinal absorp-

Table 2
The content of confiscated capsules and tablets

Code of sample	Total weight (mg)	Caffeine		pFF	
		Content (mg)	Weight (%)	Content (μg)	Weight (‰)
Capsules					
D950993	92.6	25.6	27.7	34.7	0.38
D951099A	188.4	55.3	29.4	70.5	0.37
D951099B	278.4	79.5	28.6	101.8	0.37
D951102A	199.2	60.1	30.2	97.5	0.49
D951102B	203.9	57.2	28.1	95.3	0.47
D951102C	190.4	56.2	29.5	79.1	0.42
Tablets					
D951100A	260.2	108.0	41.5	408.7	1.57
D951100B	253.1	100.0	39.5	396.3	1.57
D951103A	233.0	93.1	40.0	366.5	1.57
D951103B	225.8	89.7	39.7	351.5	1.56
D951101A	237.0	90.0	38.0	33.8	0.14
D951101B	257.3	95.0	36.9	35.3	0.14

tion and/or first-pass metabolism of fentanyl restricts the pharmacological activity. In vitro experiments suggested in that context, that gastrointestinal and hepatic first-pass metabolism at least could contribute significantly to the lack of toxicological symptoms after oral administration [35]. Based on an extrapolation of these data of fentanyl and the assumption that pFF is as potent, even the highest dose of pFF found in the capsules and tablets should not be lethal, when administered orally. However, the large variability of the doses observed for pFF could mislead abusers, potentially leading to multiple doses and thus overdosing [26,36].

Some literature relates the danger of fentanyls to *cis*-3-methylfentanyl (3-MF), suggesting that a fatal dose is 300 μg of 3-MF [24]. However, it must be considered that 3-MF is more potent than pFF and fentanyl. It also must be realised that in drug addict cases, overdoses associated with powders of 3-MF were consistent with indications of intravenous administration [13,17].

The presence of caffeine in the formulations could be explained as a cutting agent to dilute pFF. However, it would be of interest to know to what extent caffeine could have been added because of pharmacological reasons, especially since other ingredients to dilute pFF were present [21]. After all, caffeine may counteract some of the potential side effects caused by pFF such as bradycardia [37]. A similar case already has been reported in which small amounts of amphetamine have been added to an illicit fentanyl powder presumably in order to compensate for possible psychological depression caused by fentanyl [17]. Another reason could be just the intention of the manufacturer to add caffeine to heroine samples in order to increase the efficiency of vaporisation of heroine during smoking [38]. After all, the potential target group for fentanyls are the heroine abusers.

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