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Journal of Chromatography B, 59 (1997) 59–70

JOURNAL OF CHROMATOGRAPHY B

Gas chromatographic–mass spectrometry and gas chromatographic–Fourier transform infrared spectroscopy assay for the simultaneous identification of fentanyl metabolites

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Received 27 September 1996; revised 3 December 1996; accepted 9 December 1996

Abstract

Fentanyl, a synthetic opioid, undergoes important biotransformation to several metabolites. A gas chromatographic–mass spectrometric assay was applied for the simultaneous analysis of fentanyl and its major metabolites in biological samples. The identification of different metabolites was performed by gas chromatography–mass spectrometry (electronic impact and chemical ionisation modes) and gas chromatography–Fourier transform infrared spectroscopy. In the present study, rat and human microsomes incubation mixtures and human urines were analysed. In vitro formation of already known fentanyl metabolites was confirmed. The presence of metabolites not previously detected in human urine is described.

Keywords: Fentanyl

1. Introduction

Fentanyl (Ft I) is a synthetic opiate widely used for surgical analgesia and sedation. Ft I undergoes extensive hepatic biotransformation to metabolites coming from hydrolysis, N-dealkylation or hydroxylation reactions [1,2]. Previous studies used ion-cluster techniques to detect and identify metabolites of Ft I [3,4]. Thus ten different metabolites were already identified thanks to the analysis of several biological fluids such as blood [5], urine [3,6], saliva [7], as well as microsomes [8] or isolated hepatocytes [4].

The purpose of this work was to develop an analytical method to simultaneously detect and identify fentanyl metabolites. Gas chromatography–mass spectrometry (GC–MS) was used to described and identified Ft I metabolites in rat microsomes, while GC–Fourier transform infrared spectroscopy (GC–FTIR) was used for the first time to confirm these fentanyl metabolites identification. Then, the urine

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PII S0378-4347(97)00050-9

metabolic profile from patients prescribed long-term fentanyl therapy were compared to the metabolic profile recorded in the human or in the rat in vitro metabolism model. We also determined whether or not the identified metabolites were present in human urine since Goromaru et al. [4] suggested the presence of unknown metabolites after the observation of a low urinary recovery.

2. Experimental

2.1. Chemicals and reagents

Ft I (1-[2-phenethyl]-4-N-[N-propionylanilino]-piperidine) was kindly provided by Janssen (Beerse, Belgium). Commercial sources provided the N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA–TMCS), pyridine (Pierce, Rockford, IL, USA), dichloromethane (Merck, Darmstadt, Germany), $NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, dexamethasone, 3-methylcholanthrene, pyrazole and phenobarbital (Sigma, St. Quentin Fallavier, France).

2.2. Microsomes and incubation conditions

Three fresh human liver samples were obtained from patients who underwent a partial hepatectomy, as an excess of material was removed during surgery on the liver [9]. Adult male Sprague–Dawley rats (180–200 g) were treated with intraperitoneal phenobarbital (80 mg/kg), 3-methylcholanthrene (20 mg/kg), pyrazole (200 mg/kg), dexamethasone (100 mg/kg), or sodium chloride 0.9% (5 ml/kg) daily, for three days, to induce hepatic microsomal enzymes. On the fourth day, animals were sacrificed and the livers removed.

Microsomes were prepared as described elsewhere [10]. Microsomal incubations were carried out at 37°C for 60 min in a final volume of 2 ml containing liver microsomes (2–5 mg), 100 m*M* potassium phosphate buffer (pH 7.4), NADPH-generating system (10 m*M* glucose-6-phosphate, 1.5 IU glucose-6-phosphate dehydrogenase, 0.5 m*M* $NADP^+$), and fentanyl (0.2–1 m*M*). Reactions were initiated by the addition of the substrate, and were stopped adding 0.4 ml of 1 *M* NaOH.

2.3. Urine specimens

The subjects of the study were seven patients from an intensive care unit who had been receiving a fentanyl infusion (0.2–0.3 μg/h) for less than three days. Medications associated with fentanyl (aminoglycoside antibiotics, β-lactam antibiotics, midazolam, dopamine, furosemide) were not analysed, but were determined not to interfere with the analytical method (data not shown). Urines for 24 h were collected in a plastic bag via a catheter. An aliquot of 20 ml was analysed.

2.4. Sample preparation and derivatization

Microsomal incubation mixtures were extracted twice by 8 ml of dichloromethane. The combined extracts were evaporated to dryness under a nitrogen stream at 40°C.

Before sample preparation, urines were centrifuged for 10 min at 3000 *g* to eliminate particles in suspension. The chemical hydrolysis of urine samples was realised as follows: the pH of the urine was adjusted to 2.0 with 2 *M* sulphuric acid. The mixtures were then transferred into small glass bottles tightly sealed with lined caps and placed into a thermostated bath at 100°C. After 3 h of incubation, the urines were cooled in cold water at room temperature and the pH adjusted to 12.0 with 10 *M* NaOH. The urines were then extracted twice by 15 ml of dichloromethane. The extracts were evaporated to dryness under a gentle stream of nitrogen at 40°C. The resulting analytical samples (microsomes and urines) were reconstituted in 40 μl of dichloromethane.

The urines were also treated with hydrolytic enzymes (β-glucuronidase/arylsulphatase) according to the previously described method [11].

Trimethylsilyl derivatives were prepared by reacting the dichloromethane solution with 35 μl of BSTFA–TMCS in 25 μl of pyridine at 50°C for 40 min. The resulting solutions were analysed without removing the excess of derivatizing reagent, and were transferred into airtight amber glass vials.

2.5. Instrumentation

2.5.1. GC–MS

GC–MS analyses were carried out using a Hewlett-Packard 5890 Series II gas chromatograph coupled with an HP 5889A mass selective detector. The chromatographic separations were achieved on an HP-1 capillary column (25 m×0.20 mm I.D., 0.33 μm film thickness, Hewlett-Packard, Palo Alto, CA, USA). The injector and interface temperatures were set at 280°C. The oven temperature was held at 150°C for 1 min and raised up to 270°C at 10°C/min and maintained at this temperature for 8 min. It was then programmed to 300°C at 20°C/min and maintained at this temperature for 10 min. The GC–MS injected volume was 2 μl and the solutions were injected in the splitless mode. The carrier gas was helium 5.5 and the column head pressure was 20 psi at 150°C (constant pressure at 150°C, the flow-rate was 0.75 ml/min and the linear velocity of 35.8 cm/s). The mass spectrometer conditions were as follows: trap current 100 μA; ionising energy 70 eV and 230 eV for electron impact ionisation (EI) and chemical ionisation (CI), respectively. Methane was used as the reagent gas for measurements in the CI mode, and the source pressure was adjusted to 2×10^{-4} Torr.

2.5.2. GC–FTIR

GC analyses were performed on a Perkin-Elmer 8500 gas chromatograph equipped with an HP-1 capillary column (25 m×0.32 mm I.D., 0.33 μm film thickness) and the FTIR spectra were recorded on a Nicolet 20SXC FTIR spectrometer (Trappes, France). The same chromatographic and injection conditions were used for both GC–MS and GC–FTIR analyses. The spectra were recorded at an 8 cm^{-1} resolution. The spectra were obtained in transmittance mode. Scans were routinely performed from 4000 to 650 cm^{-1} at a scan rate of one spectrum per second. The transfer line temperature was set at 290°C. The volume of the glass borosilicated light pipe was 120 μl. A FTIR spectrometer (Model 5965B, Hewlett-Packard) coupled on-line with a mass spectrometer (Model 5971A, Hewlett-Packard) was used to establish the correspondence between the mass spectrum and the infrared spectrum for the same chromatographic peak. MS and FTIR conditions were similar to those above mentioned.

3. Results and discussion

First, a large amount of Ft I was incubated with microsomes from untreated rat to obtain substantial quantities of the various metabolites in order to clearly separate and identify them. Simultaneous GC–MS analyses of all metabolites (EI and CI modes) were performed on this mixture, and the results were compared with those previously described by several authors. Then to confirm the identification of several metabolites, for the first time, GC–FTIR was employed. Second, rat and human microsomes as well as human urines were analysed by GC–MS. Extracts from human urines showed many interfering peaks on GC chromatograms, and the detection of the metabolites by GC–FTIR was extremely hypothetical.

3.1. Metabolites identification by GC–MS and GC–FTIR

3.1.1. Simultaneous identification of metabolites by GC–MS

The structures of different metabolites of Ft I previously identified in biological samples are summarised in Fig. 1. Briefly, in EI mode, when an hydroxylation occurs on the phenethyl group the mass spectra present m/z 245 and m/z 189 ions. In the same way, if an hydroxylation occurs on the piperidine ring or on the propionyl moiety, the mass spectra present principal ions at m/z 333 and m/z 189. Finally, the mass spectra of N-dealkylated metabolites display m/z 155 and m/z 247 ions. Extracted ion chromatograms of m/z 155, 245 and 333 ions obtained under EI are shown in Fig. 2. These ions were chosen because they correspond to the previously reported major fragments of fentanyl metabolites [3,4,6]. Moreover, the m/z values of the ions observed in the mass spectrum of each chromatographic peak are gathered in Table 1.

Peaks a–j were not present in a blank incubation mixture with the exception of peak d which corresponds to Ft I. With increasing incubation times the peak of Ft I progressively decreased while the other

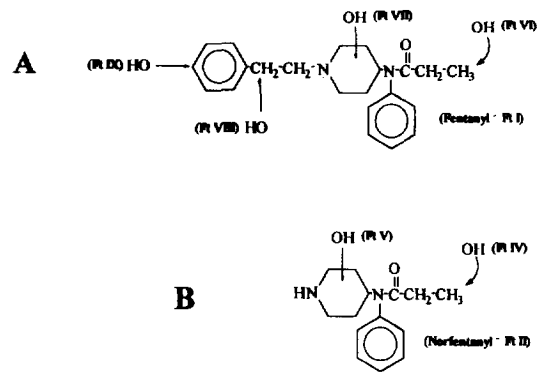

Fig. 1. Structure of fentanyl (Ft I) and its metabolites. Corresponding peaks in chromatograms of Fig. 2 are indicated in brackets. (A) Introduction of hydroxyl group into Ft I on propionyl moiety (Ft VI–peak f), on piperidine ring (Ft VII–peak h), on *alpha* position of phenetyl group (Ft VIII–peak e) and on *para* position of phenyl ring (Ft IX–peak j). (Compounds resulting from dihydroxylation of Ft I (peaks g and i) were not presented). (B) Introduction of hydroxyl group into Ft II (norfentanyl–peak a) on propionyl moiety (Ft IV–peak b) and on piperidine ring (Ft V–peak c).

peaks increased. Thus, it was possible to postulate that the compounds detected as peaks a–j are metabolites of Ft I (except for peak d).

The mass spectrum corresponding to peak a is similar to that previously described for the trimethylsilylated derivative of 4-N-(N-propionylanilino)-piperidine (Ft II) [3]. So peak a can be attributed to this metabolite. For peaks b and c, the estimated molecular mass is 392. This was determined from the $[M+H]^+$ ion (m/z 393), adduct ions [M+29] (m/z 421) and [M+41] (m/z 433) detected in CI mode, the $M^{+\cdot}$ ion (m/z 392) and the [M−15] ion (m/z 377) recorded in EI mode. This value is 88 a.m.u. higher than that of Ft II and indicates the possible introduction of a trimethylsilyl-ether group (O-TMS) in the Ft II structure. Moreover, peaks a, b and c present common fragments ions suggesting similar chemical structures. The mass spectra corresponding to peaks b and c are similar to those previously described [3]. Consequently, peaks b and c can be respectively identified as trimethylsilylated derivatives of 4-N-(N-hydroxy-propionylanilino)-piperidine (Ft IV) supported by the m/z 117 ion assigned to $[CH_2\text{-}CH_2\text{-O-TMS}]^+$ and 4-N-(N-propionylanilino)hydroxypiperidine (Ft V).

Ft I was identified as peak d by comparing its retention time and mass spectrum with those of the authentic compound. The major fragments of the spectrum are represented by the ions at m/z 245, m/z 189 and m/z 146 according to the fragmentation pathway of Ft I described by Goromaru et al. [4].

The molecular mass of compounds corresponding to peaks e, f, h and j was determined to be 424 from the $[M+H]^+$ ion (m/z 425), adduct ions [M+29] (m/z 453) and [M+41] (m/z 465) observed in CI mode and the [M−15] ion (m/z 409) observed in EI mode. This value is 88 a.m.u. higher than that of Ft I and indicates the possible introduction of an O-TMS in the structure of Ft I. The main fragments ions of these four peaks are gathered in Table 1. GC–MS observations strongly suggest that peaks e, f, h and j are metabolites of Ft I and this is confirmed by GC–FTIR analyses (cf. Section 3.1.2.).

The fragment ion of highest m/z (m/z 333) corresponds to the elimination of the $C_6H_5\text{-}CH_2$ group (91 a.m.u.) from molecular ion (m/z 424) of f and h compounds. Moreover, the presence of an ion at m/z 117 (assigned as $[CH_2\text{-}CH_2\text{-O-TMS}]^+$) in f spectrum, suggest that respectively an hydroxylation occurs on the propionyl moiety and on the piperidine ring of Ft I resulting in the formation of f and h compounds. The mass spectra of these two peaks are similar to those previously described and suggest that peaks f and h respectively correspond to 1-(2-phenethyl)-4-N-(N-hydroxy propionylanilino)-piperidine (Ft VI) and 1-(2-phenethyl)-4-N-(N-propionylanilino)hydroxy piperidine (Ft VII) [4].

Mass spectra (EI and CI) of peaks e and j are similar and close to the fentanyl spectrum obtained under EI. This observation suggests that an hydroxylation takes place on the phenethyl group of Ft I as previously described [4]. However, the mass spectra data do not supply enough information to clearly elicit the structure of both compounds.

Compounds corresponding to peaks g and i have a molecular mass determined at 512 from the $[M+H]^+$ ion (m/z 513) observed in the CI mode and the [M−15] ion at m/z 497. This value corresponds to an increase of 176 a.m.u. from Ft I and suggests the introduction of two hydroxyl groups into the Ft I structure. For the compound corresponding to peak g, ions at m/z 333 and 117 point out that one hydroxylation had occurred on the propionyl moiety

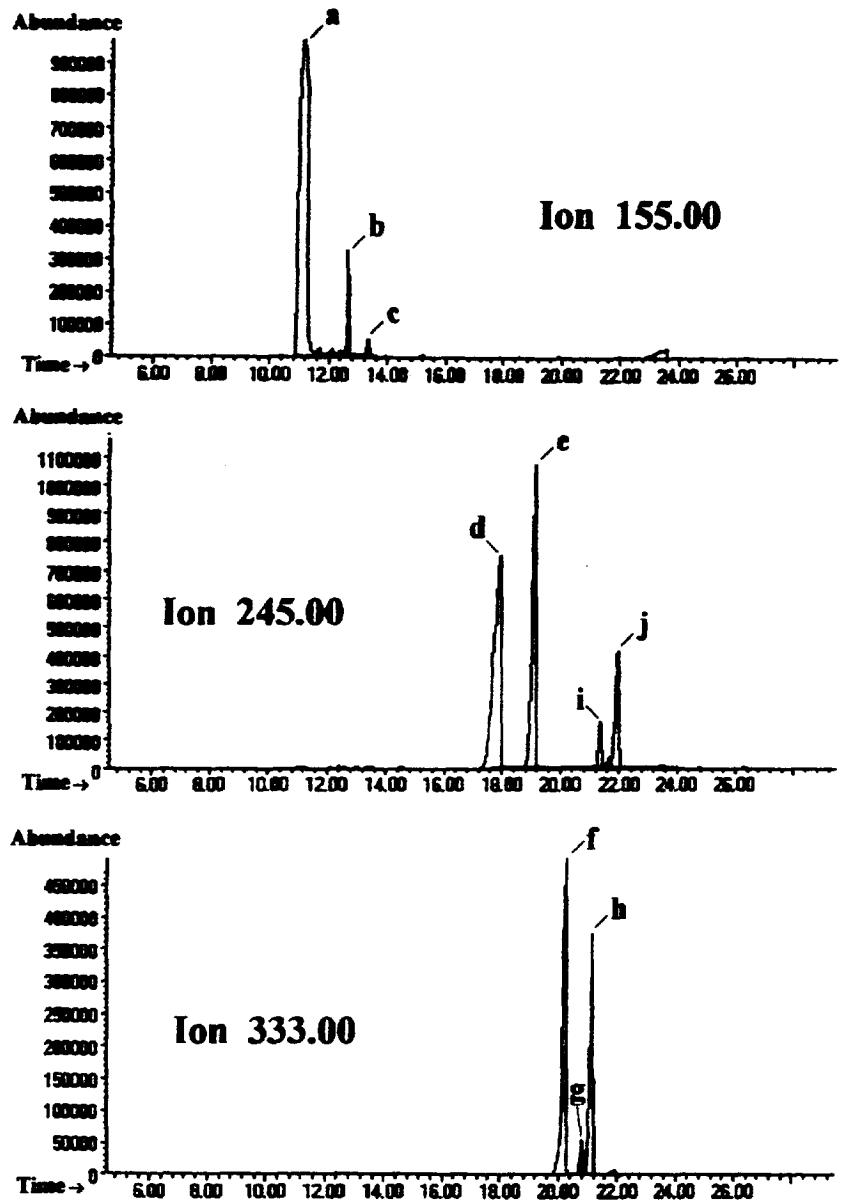

Fig. 2. Selected ion chromatograms of rat microsomes incubation mixture. The monitored ions were (*m/z*) 155, (*m/z*) 245, (*m/z*) 333. Peaks appear at 11.12 min (peak a), 12.65 min (peak b), 13.34 min (peak c), 17.98 min (peak d), 18.16 min (peak e), 20.26 min (peak f), 20.93 min (peak g), 21.18 min (peak h), 21.35 min (peak i), 21.99 min (peak j).

and the other one on the phenethyl group. The mass spectrum (EI mode) of peak i shows the same fragment ions as Ft I and its metabolites e and j. These observations suggest that two hydroxyl groups have been introduced into the phenethyl group.

The present analytical conditions allowed the detection of nine out of the ten described metabolites of Ft I [3,4,6]. Only Ft III [1-(2-phenethyl)-4-N-anilinopiperidine or despropionylfentanyl] identified elsewhere in rat liver tissue [1] and in human plasma [5] was not detected in the present study. Due to the lack of authentic Ft III standard, it was impossible to

Table 1
Mass spectral data (relative abundances to base peak–%) of the compounds detected from *m/z* 155, 245, 333 extracted chromatograms (in rat microsomal incubation)

Peak	*m/z* Values of observed ions																				
	Electronic impact																	Chemical ionisation			
	73	117	146	155	189	202	206	231	245	247	275	289	304	310	333	377	392	409	M+1	M+29	M+41	M−15
a	100			98			20	15		56		289	17						305	333	345	nd
b	98	25		100						71	10			4		12	5		393	421	433	377
c	44			100				6		32						7	3		393	421	433	377
d			90		65	20			100										337	365	377	nd
e	40		60		50	20			100									3	425	453	465	410
f	100	95	30		70										90			5	425	453	465	410
g	100	22	66		60										60				513	nd	nd	497
h	45		70		100										98			5	425	453	465	410
i	44		48		36	12			100										513	541	nd	497
j	25		53		50	18			100									3	425	453	465	410

nd: not detected. The relative abundances of detected molecular ions are underlined.

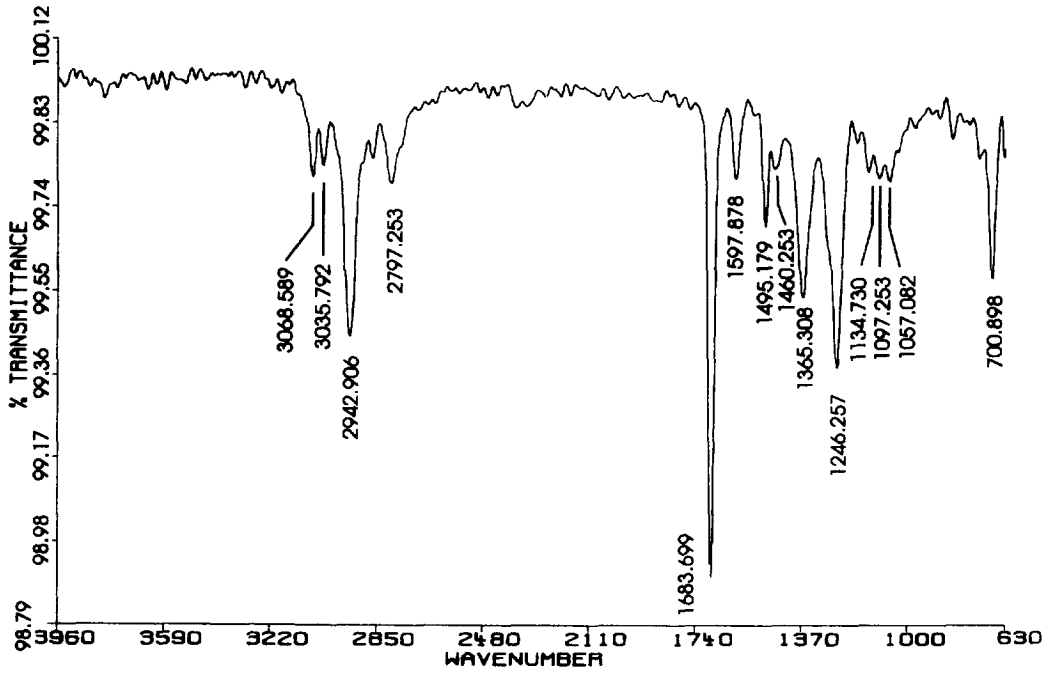

Fig. 3. Fourier transform infrared spectrum of fentanyl. GC–FTIR conditions are described in Section 2.5.2

test our analytical procedure for Ft III detection. However, our results are in good agreement with previous studies in which Ft III was neither detected from isolated hepatocytes [4] nor from human urine [6,7].

3.1.2. Identification of several fentanyl metabolites by GC–FTIR

The FTIR spectrum of fentanyl is shown in Fig. 3. The main band at 2942 cm^{-1} clearly arises from the C–H bonds in a saturated hydrocarbon. The smaller peak at 2797 cm^{-1} close to the main band of the spectrum is probably due to C–H symmetrical stretching in a methyl group. The peak at 1460 cm^{-1} probably arises from the bending of the $-CH_2-$ groups mentioned above. The presence of aromatic structures is pointed out in various spectral regions: 3068 and 3035 cm^{-1} for the =C–H stretching vibration, the group of bands between 1595 and 1495 cm^{-1} correspond to the region where skeletal vibrations in aromatic structures give characteristic absorptions. The absorption band at 700 cm^{-1} can be assigned to out-of-plane vibrations of aromatic C–H groups. However in this region, the interpretation needs some caution due to the less specific character of absorption bands. The presence of a tertiary amide group is shown by a combination of bands: one with high intensity at 1683 cm^{-1} corresponding to $\nu_{C=O}$ and the other one with medium intensity at 1246 cm^{-1} corresponding to ν_{C-N}. The absorption band at 1365 cm^{-1} can be assigned to the stretching vibration of the C–N group of a tertiary amine substituted by an aromatic ring.

The FTIR spectra recorded on e, f, h and j peaks show the main characteristic bands of Ft I, suggesting that these compounds structures present methyl group, aromatic structures, tertiary amide and tertiary amine.

The FTIR spectra of peaks f and h are shown in Figs. 4 and 5. In both cases, an intense band in the 1150–1100 cm^{-1} region clearly arises from the Si–O–C bonds of the O-TMS. Moreover, the presence of a trimethylsilyl group is confirmed by absorption bands at 850 cm^{-1} and 750 cm^{-1} attributed to stretching vibrations of $Si-(CH_3)_3$ groups.

Some bands of the peak j infrared spectrum provide useful information on the probable location of the hydroxyl group in the molecule structure (Fig.

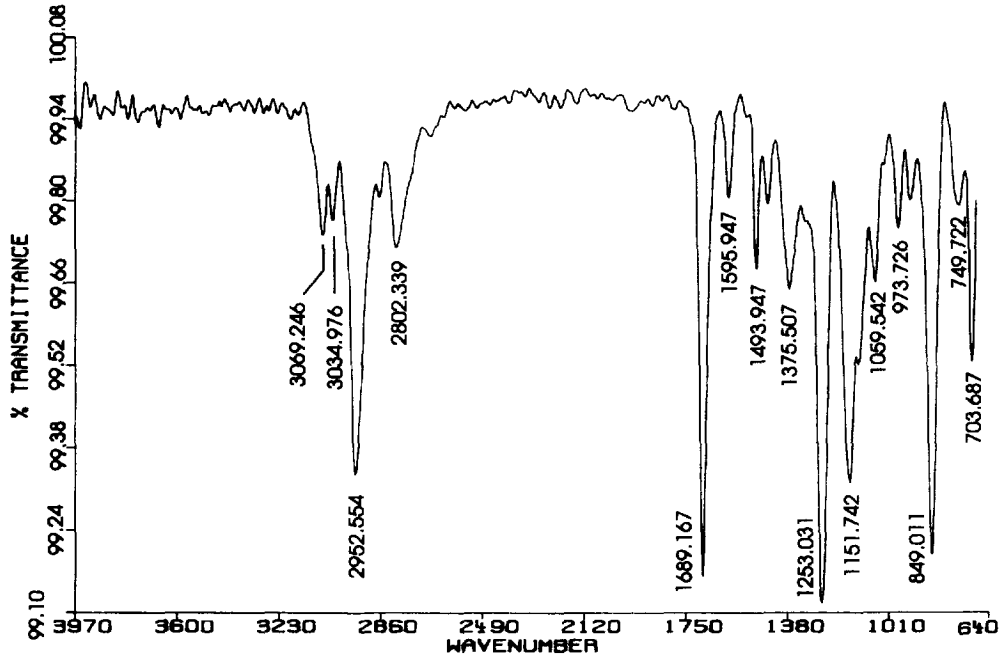

Fig. 4. Fourier transform infrared spectrum of peak f. GC–FTIR conditions are described in Section 2.5.2.

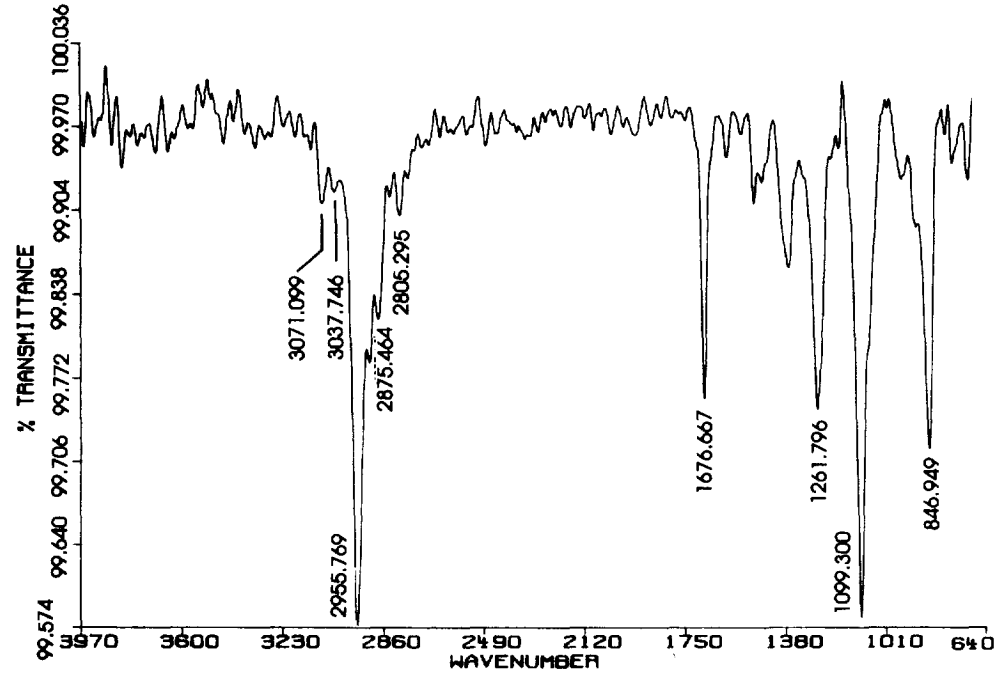

Fig. 5. Fourier transform infrared spectrum of peak h. GC–FTIR conditions are described in Section 2.5.2.

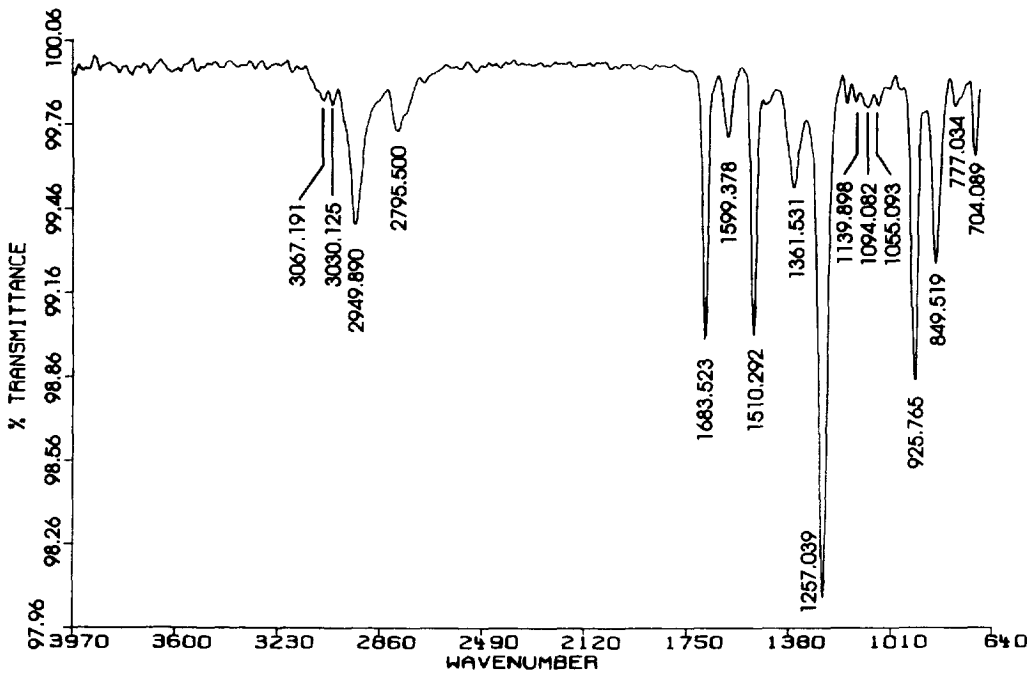

Fig. 6. Fourier transform infrared spectrum of peak j. GC–FTIR conditions are described in Section 2.5.2.

6). The medium intensity band at 925 cm^{-1} can be assigned to stretching vibration of Si–O–aromatic group suggesting that an hydroxylation occurs on the phenyl ring. The band at 1510 cm^{-1} can be observed for ν_{CC} when an aromatic ring is substituted in *para* with an electrondonor group such as O-TMS [12]. Moreover, the presence of the intense band at 1257 cm^{-1} may be ascribed to the superimposition of the ν_{C-N} band from the amide group and the Si–O–C stretching band of the O-TMS which was shifted from 1100 cm^{-1} to 1250 cm^{-1} due to the *para* substitution. So peak j can be attributed to 1-(2-*para*hydroxyphenethyl)-4-N-(N-propionylanilino)piperidine (Ft IX).

The infrared spectrum corresponding to peak e (Fig. 7) is qualitatively close to those of Ft VI and Ft VII and does not display the characteristic absorption bands of aromatic substitution as mentioned above for peak j. Data from mass and infrared spectra indicate the possible introduction of an hydroxyl group in *alpha* position of the phenethyl group, and suggest that peak e corresponds to 1-(1-hydroxy-2-phenethyl)-4-N-(N-propionylanilino)piperidine (Ft VIII).

The compounds corresponding to the peaks g and i could not be observed by GC–FTIR due to their relative weak level in the samples.

3.2. Applications of the GC–MS method

3.2.1. Metabolite profiles from human and rat microsomes

Rats were induced to selectively increase the microsomal content of cytochromes P-450 monooxygenases (CYP450) 1A (3-methylcholanthrene), 2B (phenobarbital), 2E1 (pyrazole) and 3A (dexamethasone). In all cases, Ft II (norfentanyl) formed by a N-dealkylation reaction at the piperidine nitrogen atom of Ft I was found as the main metabolite. The relative proportions of the other metabolites were different according to the markedly enhanced CYP450 isoform after chemical induction (Table 2). These data bring out the relative specificity of metabolite formation according to the CYP450 isoforms involved.

As described above for rats, the results from three human microsomes samples showed Ft II as the main

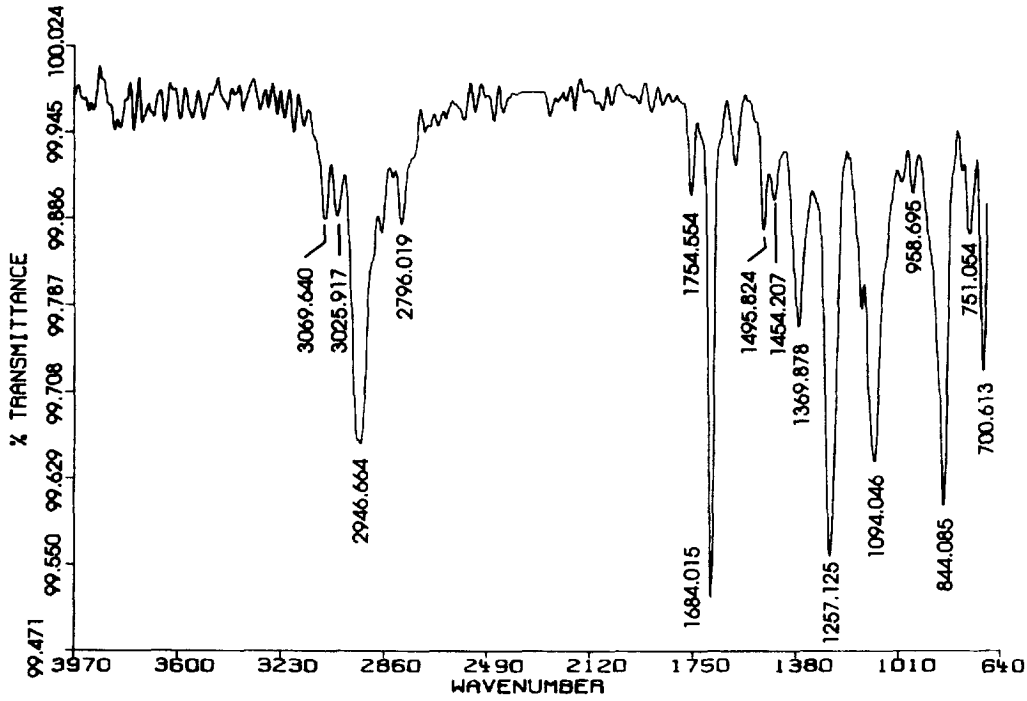

Fig. 7. Fourier transform infrared spectrum of peak e. GC–FTIR conditions are described in Section 2.5.2.

metabolite and at a lower level the presence of Ft IV, Ft VI and Ft VIII.

Results from rat and human microsomes were in good agreement with previous studies, where N-dealkylation has been described as the major metabolic pathway for Ft I [2,6], as well as for alfentanil [13] and sufentanil [14] two other synthetic opioids, structurally close to Ft I.

3.2.2. Metabolite profiles from human urines

The occurrences of Ft I and its detected metabolites in the urines of seven patients are presented in Table 3. Ft I and Ft II were identified in the urine of all examined patients. These data confirm on the one hand that a fraction of unchanged Ft I is excreted in urine and on the other hand that Ft II is the main metabolite of Ft I detected by our method. Neverthe-

Table 2
Detection of fentanyl metabolites from rat microsomes after chemical induction

Rat treatment	Ft II (peak a)	Ft IV (peak b)	Ft VI (peak f)	Ft VII (peak h)	Ft VIII (peak e)	Ft IX (peak j)
None	+++++	+/−	++	++	++	++
Dex.	++++++	+++	nd	nd	nd	nd
3-MC	+++++	+/−	++	+	++	++++
PB	++++++	+++	++	+/−	+/−	+/−
Pyr.	++++	+/−	+	+	++	++

Proportion for each metabolite was expressed in comparison with the peak area of fentanyl at 100 μ*M* (corresponding to six crosses). PB: phenobarbital. 3-MC: 3-methylcholanthrene. Pyr.: pyrazole. Dex.: dexamethasone. Incubation conditions are described in Section 2.2. nd: not detected.

Table 3
Detection of fentanyl metabolites in patient's urines

Patient	Ft I (peak d)	Ft II (peak a)	Ft IV (peak b)	Ft V (peak c)	Ft VI (peak f)	Ft VII (peak h)	Ft VIII (peak e)	Ft IX (peak j)	UM
1	+	+	–	–	–	+	–	+	+
2	+	+	–	–	–	+	+	–	+
3	+	+	–	–	–	–	+	–	+
4	+	+	–	–	–	+	+	–	+
5	+	+	–	–	–	–	–	–	+
6	+	+	–	+	–	–	–	–	+
7	+	+	–	–	–	+	+	+	+

UM: unknown metabolite; +: detected; –: not detected.

less, confirming our observations, Ft II has been previously described as the main metabolite of Ft I [2,4]. Ft VIII and Ft IX were previously identified in isolated hepatocytes from rat and guinea pig, and Ft IX was identified in urine from both species [4]. In contrast to the literature, Ft V, Ft VII, Ft VIII and Ft IX were detected in our human urine samples [6]. However, Ft IV and Ft VI were not detected in any of the examined urines although these two metabolites have been already identified in human urine [6]. This lack of detection may be due either to loss during the extraction procedure, particularly during the chemical hydrolysis, or to the absence of these metabolites in the original samples.

From the urines of the seven examined patients, a compound which displays an EI mass spectrum close to that of Ft I but different from those already observed was detected. This peak was also observed when urines were treated with β-glucuronidase/arylsulphatase (*Helix Pomatia*) but was not detected

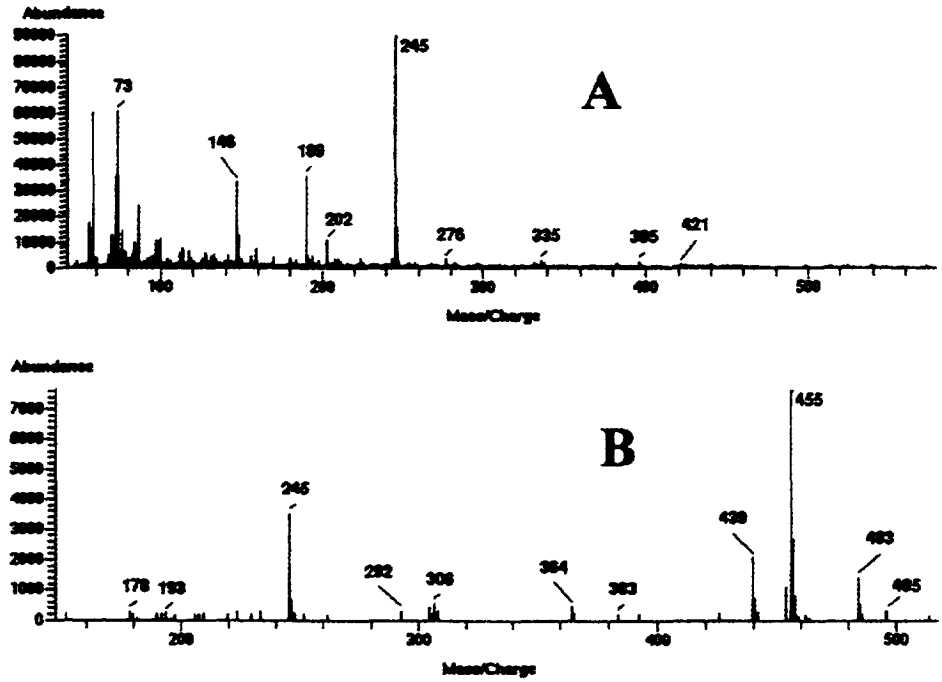

Fig. 8. Mass spectrum of peak corresponding to the compound detected in all human urines in EI mode (A) and in CI mode (B) at a retention time of 21.72±0.16 min ($n=7$).

in urines analysed before hydrolysis. It has been verified that urines treated with hydrolytic enzymes gave the same results as urines treated with strong acid. Thus, it appears that the fentanyl metabolites are stable in this way. The molecular mass was determined at 454 from the $[M+H]^+$ ion (m/z 455), adduct ions [M+29] (m/z 483) and [M+41] (m/z 495) observed in CI mode and the [M−15] ion (m/z 439). Consequently the value was shifted by 118 a.m.u. from Ft I value (Fig. 8).

According to the metabolic pathway of Ft I biotransformation, it is possible to suggest the introduction of O-TMS (adding 88 a.m.u.) and of a methoxy group (adding 30 a.m.u.) in the structure of Ft I, suggesting that this compound might be an hydroxy–methoxy metabolite of Ft I. According to our previous observations from EI mass spectra of compounds substituted on the phenethyl group such as Ft VIII, Ft IX or dihydroxylated metabolite (peak i) which display similar mass spectra, the O-TMS and methoxy substitutions might occur on phenethyl group. The hypothesis, of hydroxy–methoxy metabolites of Ft I in urine was revealed by Goromaru et al. who found this metabolite in the guinea pig urine. However, the authors did not supply any structural or spectral data of this compound [4].

The postulated metabolic pathway leading to this metabolite involves a dihydroxylation on the phenethyl group (phase I reaction) followed by a glucuroconjugation and an O-methylation (phase II reaction). The absence of detection in in vitro preparations was expected since its formation requires cathecol-O-methyltransferase which is not a microsomal enzyme. These data confirm that hydroxylation of the phenethyl group is one of the main metabolic pathways of Ft I [4].

4. Conclusion

In contrast to previous works, a GC–MS assay for the simultaneous detection of the main fentanyl metabolites and applied to in vitro and in vivo studies is described. Due to the lack of authentic metabolites standards of Ft I or deuterium labelled Ft I, the identification of the metabolites was based on MS and for the first time, on FTIR. Data obtained from both techniques allowed the identification of nine out of the ten described metabolites of Ft I.

These analytical methods allowed the identification of new human in vivo metabolites. Indeed, for the first time these compounds were identified in human urines whereas they were previously only detected in vitro or in urines of other species.

Acknowledgments

The authors are grateful to Mrs A. Leroux for her technical assistance, Mr Bartholin for his generous support and Professor Longeray for helpful discussion on infrared spectroscopy.

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