

Case report / Étude de cas

Buprenorphine and norbuprenorphine determination by GC/MS in putrefied biological samples: evaluation of a buprenorphine related death

Dosages par CG/SM de la buprénorphine et de la norbuprénorphine dans des prélèvements biologiques en décomposition : étude d'un décès lié à la buprénorphine

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Abstract – Introduction: Deaths related to buprenorphine are rarely discovered in Italy. This paper describes a case report regarding a young marine that was found dead, in an advanced state of decomposition, in which buprenorphine/norbuprenorphine (BUP/norBUP) are identified and quantified by GC/MS. **Material and methods:** BUP/norBUP were extracted from hair and from putrefied samples after acid hydrolysis (hair) and enzymatic hydrolysis by β -glucuronidase (blood, liver, urine and bile). Instrumental identification was carried out on GC/MS (Agilent 5973N) (SIM mode), monitoring respectively 450–482–506 (m/z) ion for buprenorphine and 468–500–524 (m/z) ion for nor-buprenorphine, compared to certified materials and deuterated internal standards. **Results:** In spite of putrefaction the presence of BUP/norBUP was demonstrated in liver (8.4 ng/mg); blood (24 ng/mL); urine and bile positive. In the hair, beyond BUP/norBUP, they were discovered cocaine, BEG, 6-MAM and morphine. Complementary systematic analyses by GC/MS revealed clozapine, lormetazepam and amoxapine, only in urine and bile. All these data support the hypothesis that the subject was under buprenorphine substitution treatment with contemporary use of antidepressive and tranquilizer drugs. **Conclusion:** These data demonstrate the persistence of BUP/norBUP in post mortem samples and contribute to highlight the incidence of buprenorphine in deaths involving addicts of heroin and other psychotropic drugs.

Key words: Buprenorphine, norbuprenorphine, putrefied samples

Résumé – Introduction : Les décès liés à la buprenorphine sont rares en Italie. Cet article décrit un cas concernant un jeune marin retrouvé mort, dans un état de décomposition avancé, avec identification et dosage par CG/SM de la buprenorphine et de la norbuprenorphine (BUP/norBUP). **Matériels et Méthodes :** BUP/norBUP ont été extraits des cheveux et des prélèvements putréfiés, après hydrolyse acide (cheveux) et hydrolyse enzymatique par la β -glucuronidase (sang, foie, urine et bile). La détection par CG/SM a été faite en mode SIM (m/z 450–482–506 pour la buprenorphine, m/z 468–500–524 pour la nor-buprenorphine), et comparaison à des produits certifiés, avec des étalons internes deutérés. **Résultats :** Malgré la décomposition, BUP/norBUP ont été détectées dans le foie (8,4 ng/g), le sang (24 ng/mL), l'urine et la bile. Dans les cheveux, outre BUP/norBUP, de la cocaïne, de la BEG, de la 6-MAM et de la morphine ont été détectées. Des analyses de routine complémentaires par CG/SM ont révélé la présence de clozapine, lormétazépam et amoxapine dans l'urine et la bile uniquement. Toutes les données étayent l'hypothèse que le sujet était sous traitement de substitution à la buprénorphine, avec prise concomitante d'antidépresseurs et de tranquillisants. **Conclusion :** Ces résultats démontrent la persistance de BUP/norBUP dans les prélèvements post mortem, et mettent en perspective le rôle de la buprénorphine dans la survenue de décès de personnes dépendantes à l'héroïne et autres substances psychotropes.

Mots clés : Buprénorphine, norbuprénorphine, prélèvements putréfiés

Received 9 June 2008, accepted after revision 5 February 2009
Published online 19 March 2009

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1 Introduction

Addiction or deaths related to buprenorphine (BUP) are rarely discovered, in Italy, even though this drug has been widely using, since 2000, as substitution treatment for heroin addicts, instead of Methadone. The drug is metabolized to norbuprenorphine (norBUP) and subsequently conjugation with glucuronic acid of both buprenorphine and the N-desalkyl metabolite occur but very low levels in biological fluids are recovered after therapeutical doses or abuse.

This drug indeed is not revealed by screening methods (Emit, FPIA, EIA) routinely applied for toxicological controls of the living subjects. Moreover a specific method for buprenorphine research on corpse are performed only if circumstance of death are strongly suggestive of buprenorphine abuse. This paper describes a case report where buprenorphine and norbuprenorphine are identified and quantified in putrefied biological samples by GC/MS, in order to demonstrate the persistence of BUP/norBUP in post mortem samples and contribute to highlight the incidence of buprenorphine in deaths involving heroin and other psychotropic drugs addicts.

2 Case report

A young marine (USA) was found dead, in his residence, in an advanced state of decomposition, after a period of twenty day holiday. At inspection of the death scene were found the following pharmaceutical blisters: Subutex (8 mg), Minias (2 mg), Zolpidem (5 mg). There were neither syringes nor violence evidences. Because putrefaction, autopsy not revealed typical signs of acute narcotism, such as cyanosis, pulmonary oedema, visceral congestion. Furthermore liver, blood, hair were collected. Moreover the few liquids from the bladder and the gall-bladder were taken washing them by physiological water.

3 Materials and methods

Buprenorphine/norbuprenorphine were assayed in post mortem putrefied samples according to a methodology validated in our laboratory.

Hydrolysis :

- Blood (3 mL), liver (1g), urine and bile washing (5 mL), after addition of Nalorphine (200 ng) as internal standard, were incubated with β -glucuronidase (Helix Pomatia 100 U/mL), at pH 7 and 55 °C for 1.5 h.

- Hair pulverized (100 mg) has been treated with HCl 0.1 M (1 mL) at 55 °C overnight, after decontamination in methylene chloride and the addition of buprenorphine-d₄ and Norbuprenorphine-d₃ (10 ng).

Extraction :

Buprenorphine/norbuprenorphine from putrefied samples (blood, liver, urine and bile) and hair, were extracted by Toxi-Lab Tubes™ "A". After agitation and centrifugation, the organic phase was removed and evaporated. Dry extracts were

Table I. Buprenorphine and norbuprenorphine in putrefied samples.

Biological samples	buprenorphine	Norbuprenorphine
Blood (ng/mL)	24.0	9.6
Liver (ng/mg)	8.4	1.2
Urine	Pos.	Pos.
Bile	Pos.	Pos.

reconstituted in 50 μ L of BSTFA + 1% TMS, from which 3 μ L were injected in GC/MS.

Instrumental Analysis :

Instrumental identification was carried out by Agilent GC/MS 5890/5973N equipped with a MS₅ capillary column (30 m \times 0.25 i.d.) operating in SIM mode.

Calibration curves were prepared by certified materials (blood and urine from Medicchem Steinenbronn – Germany) containing respectively BUP/norBUP (20/80 ng/mL) in urine and 13 ng/mL for both substances in blood.

For all biological samples and certified materials were monitored the following ions (*m/z*):

buprenorphine: **450**–482–506
 norbuprenorphine: **468**–500–524
 nalorphine: **455**–414–440
 buprenorphine-d₄: **454**–486–510
 norbuprenorphine-d₃: **471**–503–527

Calibration curves were linear from 0.1 to 50 ng/mL in biological samples and from 0,01 to 4 ng/mg in hair. Under these analytical conditions the limit of quantitation (LOQ) for BUP/norBUP were respectively 0.5/0.2 ng/mL for biological samples and 0.1/0.05 ng/mg in hair.

In addition, systematic toxicological analysis of all biological samples (liver, blood, hair and diluted urine and bile) was performed using screening immunoassay (Emit) and GC/MS for pharmaceuticals and other drugs of abuse.

4 Results

In spite of the advanced putrefaction and dilution, as regards of urine and bile, the presence of buprenorphine and norbuprenorphine was demonstrated in each biological samples as shown in Table I. Complementary systematic analysis in GC/MS, revealed clozapine, lormetazepam and amoxapine, in diluted urine and bile only.

Hair results for BUP/norBUP and other drugs obtained from proximal and distal segments, showed in Table II, define a chronic drug abuser and suggest a decrease in heroin and cocaine intake. These data support the hypothesis that the subject was under buprenorphine substitution treatment with contemporary use of antidepressant and tranquillizer drugs.

5 Conclusion

International literature reports survey about deaths involving buprenorphine, where dosages in biological samples, obtained in LC/MS or GC/MS, are presented [1, 3, 7, 8] pointing out the frequent mixture of buprenorphine with sedatives,

Table II. Buprenorphine/Norbuprenorphine and other drugs in hair (ng/mg).

Hair	buprenorphine	norbuprenorphine	cocaina	BEG	6-MAM	morphine
Proximal (0–3 cm)	12.7	5.9	21.87	2.73	102.18	24.32
Distal (3–6 cm)	49.8	< LOD	69.42	5.30	320.15	78.65

neuroleptics, antidepressants and other antipsychotics drugs, or alcohol. Difficulty to evaluate the toxicological data related to therapeutic, toxic and lethal levels is also discussed [2, 4–6]. Moreover it is important to emphasize that the concentrations of BUP/norBUP are very rarely published in putrefied samples. Therefore our toxicological results, that we evaluated as therapeutic levels, may be affected by environmental conditions and other factors related to corpse (re-circulation of blood within the vessels under the action of gases of putrefaction; intra and extra cellular exchanges from organs and tissue compartments; development of microbial flora and contamination of blood from the digestive tract). All these factors represent a strong obstacle to the interpretation of the precise role of the buprenorphine and other psychotropic drugs in death process.

Nevertheless our data confirm that putrefaction doesn't a relevant interference in identification and dosage of buprenorphine/norbuprenorphine in biological materials and contribute to highlight the incidence of buprenorphine in deaths involving addicts of heroin and other psychotropic drugs in Italy.

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