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Short communication

Determination of remifentanil in human blood by capillary gas chromatography with nitrogen-selective detection

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Abstract

A validated method for the determination of remifentanil in human blood, applicable to all therapeutic concentrations, using capillary GC with nitrogen-specific detection and fentanyl as the internal standard has been developed. Citrated whole blood samples were extracted into 1-chlorobutane following precipitation of proteins with methanol. The drugs were back extracted into 10 m*M* HCl and re-extracted into methanol–1-chlorobutane. The extracts were reconstituted in methanol and injected onto a 25-m BPX-5 column. The lower limit of quantitation was 0.2 ng/ml with within- and between-day coefficients of variation of less than 15%. © 2002 Published by Elsevier Science B.V.

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1. Introduction

Remifentanil (Fig. 1) is a pure μ-opioid receptor agonist, that produces analgesia in a dose-dependent manner [1], with a terminal half-life of 15 min regardless of the duration of infusion [2]. This rapid offset of remifentanil is the result of the presence of a methyl propanoate ester group in its structure, which is highly susceptible to hydrolytic cleavage via non-specific tissue and plasma esterases. This susceptibility to ester hydrolysis makes it a much more difficult compound to assay than other similar opioids, such as alfentanil. In addition, the therapeutic blood concentrations of remifentanil, ~5 ng/ml [3], are ~20 times lower than alfentanil blood concentration at equipotent doses.

To date, bioanalytical literature for the chromatography of remifentanil has described a sensitive capillary gas chromatography method, coupled to high resolution mass spectrometry in the selected ion monitoring mode (GC–HRMS–SIM) [4], and HPLC methods with UV detection [5,6]. Although effective and robust with a limit of quantitation of 0.1 ng/ml, the GC–HRMS–SIM method requires equipment which is not always available, particularly in smaller laboratories. The HPLC methods are simpler and relatively less expensive, but have limited sensitivity at around 1–2 ng/ml. This makes the HPLC method inappropriate for the infusion pharmacokinetic studies of remifentanil in humans, as the limit of detection of the assay is within the range of con-

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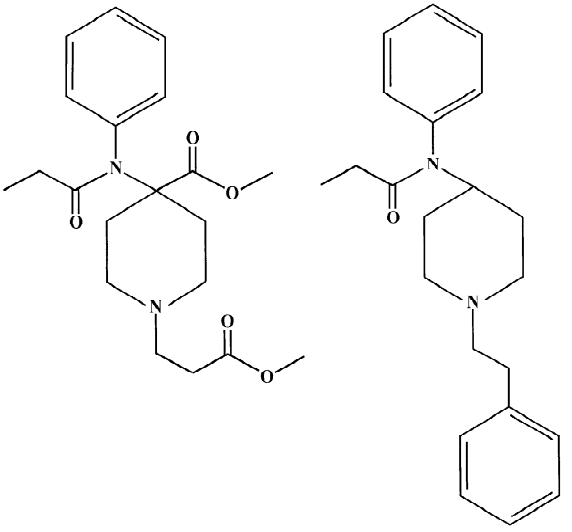

Fig. 1. Structure of remifentanil (left) and the internal standard fentanyl (right).

centrations associated with significant sedation (1–5 ng/ml). The aim of this study, therefore, was to develop and validate a sensitive, robust analytical method for capillary GC, applicable to the analysis of remifentanil at therapeutic concentrations in human blood, without requiring mass spectrometry.

2. Methods

2.1. Materials and reagents

Hydrochloric acid (HCl), citric acid, and KH_2PO_4 were purchased from Sigma (St. Louis, MO, USA). Methanol, ethanol, acetonitrile, 1-chlorobutane (HiPerSolv, BDH, Poole, UK) and pentane (Mallinckrodt, Paris, KY, USA) were of analytical grade or higher, purchased from a local supplier, and used without further purification. Purified water was obtained using a MilliQ system (Millipore, Bedford, MA, USA). Fentanyl (Sigma) was diluted with purified water for use as the internal standard. Drug-free human blood was obtained from laboratory personnel.

2.2. Instrumentation

The capillary GC system for the analysis of remifentanil consisted of a Shimadzu GC-17A gas chromatograph, with an AOC-20I auto-injector, coupled to a nitrogen-phosphorous detector, and a programmed temperature vaporizer (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). Data were transferred from the GC via a communication bus module CBM-101, to a PC running Class-GC10 software (version 1.61, Shimadzu Chemical Laboratory, Analysis System & Software, Kyoto, Japan). The BPX-5 column was 25 m×0.22 mm I.D., with a film thickness of 0.25 μm (SGE, Melbourne, Australia) using ultra-pure helium as the carrier gas (BOC Gases, Melbourne, Australia) at a linear velocity of 34 cm/s.

2.3. Preparation of stock solutions and spiked samples

Stock solutions of remifentanil at 20.0 mg/l in 2 m*M* HCl and fentanyl at 100 mg/l in methanol were stored at 4 °C in darkness. The internal standard working solution was made by dilution in water to a concentration of 2000 ng/ml. Standard samples were made by the addition of the determined quantity of stock solution to drug-free citrated blood (20 μl of 10 mg/ml citric acid per ml of blood). Standards were prepared fresh on each analysis day.

2.4. Collection of blood samples

Blood samples were collected into plain plastic blood collecting tubes containing 20 μl of 10 mg/ml citric acid per ml of blood and stored on ice before freezing at −20 °C.

2.5. Extraction of blood samples

For the extraction of remifentanil, 1.0 ml of citrated whole blood, 50 μl of fentanyl (2000 ng/ml), and 1.0 ml methanol, were added to a 15-ml polypropylene centrifuge tube. The mixture was vortex-mixed for 5 s and centrifuged for 15 min at 1000 *g*. The supernatant was transferred to a 12-ml screw-top, borosilicate glass tube containing 6 ml of 1-chlorobutane, vortex-mixed for 5 s and centrifuged for 10 min at 1000 *g*. The organic phase was transferred to a second 12-ml borosilicate glass, screw-top tube containing 200 μl of HCl (10 m*M*). The mixture was vortex-mixed for 5 s and centrifuged for 10 min at 1000 *g*. The organic (upper)

layer was discarded, the aqueous phase was washed with a further 3 ml 1-chlorobutane, which was also discarded. Methanol (1.0 ml), 1.0 ml KH_2PO_4 buffer (10 m*M*, pH 4.2), and 6.0 ml 1-chlorobutane, were added to the aqueous layer. The mixture was vortex-mixed for 10 s and centrifuged for 15 min at 1000 *g*. The organic (upper) layer was transferred to a 5-ml borosilicate glass tube, and evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted in 200 μl of methanol, vortex-mixed for 5 s, transferred to a polypropylene limited volume insert, and evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted in 20 μl of methanol, and vortex-mixed. The auto-injector was programmed to inject up to 20 μl of the sample into the programmed temperature vaporiser. The extracts were evaporated onto the injection port liner for 1 min at 65 °C followed by 1 min at 110 °C, both at a split ratio of 100:1. The split valve was closed and the injection port temperature raised at 250 °C/min to a final temperature of 270 °C with the analytical column maintained at 150 °C. Three minutes after the injection the split valve was reopened to 20:1 and the column oven temperature raised at 40 °C/min to 270 °C, which was held for 9 min.

2.6. Linearity, precision, accuracy and recovery

Six concentration levels (0.200, 0.500, 2.00, 5.00, 20.0, 100 ng/ml), each replicated six times in the one analytical run, were determined for the evaluation of within-day precision and accuracy. For evaluation of between-day accuracy and precision the same five concentration levels were determined on 6 analytical days. Absolute recovery at all six concentration levels was estimated by comparison with directly injected solutions of remifentanil in methanol.

3. Results and discussion

Representative chromatograms of a blank sample, a patient sample near the limit of quantitation, and a patient sample at a therapeutic level are shown in Fig. 2. The retention times for remifentanil and fentanyl were 9.1 and 10.9 min, respectively. Calibration curves generated using weighted ($1/y^2$) least squares regression were linear over the concentration range with correlation coefficients (r) greater than 0.995 in each case. The within- and between-day precision and accuracy details are presented in Table 1. The limit of quantitation was 0.2 ng/ml as defined by within- and between-day relative standard deviations (RSD) of less than 15%.

It was necessary to develop a new assay and extraction method for remifentanil for several reasons: (1) there was a limitation on the the availability of sophisticated GC equipment, (2) the HPLC methods [5,6] were not sufficiently sensitive for our purposes, (3) we could not reproduce the extraction into chlorobutane described in these methods, (4) the single extraction described for GC–MS [4] was too crude for use with a nitrogen-selective detector.

The major challenge in developing a sample preparation method for remifentanil lies in the presence of the methyl propanoate ester group. Not only is this linkage susceptible to enzymatic hydrolysis by plasma and tissue esterases but it also undergoes chemical hydrolysis at pH 7 and above [5]. The addition of citric acid (20 μl at 10 mg/ml per ml of blood) lowers the pH sufficiently to prevent both forms of hydrolysis, allowing blood samples to be safely stored for at least 20 h at room temperature, frozen for at least 1 year and passed through three freeze–thaw cycles without significant degradation of remifentanil [5]. This approach is simple and convenient, particularly when compared with the technique of immediate in situ precipitation of proteins with acetonitrile and extraction into dichloromethane [4].

Although we could not reliably extract remifentanil into chlorobutane alone, even when buffered to pH 7.4 [5,6], we did find that the addition of methanol, which we used in an initial protein precipitation step, to the chlorobutane produced quantitative extraction even at the pH of the citrated blood ($pH \cong 4$). This eliminated the need to buffer the samples back into the pH range where ester hydrolysis occurs, removing the potential for remifentanil breakdown during the extraction procedure.

The programmed temperature vaporiser used in our GC instrument was operated in a manner similar in effect to the traditional splitless technique. How-

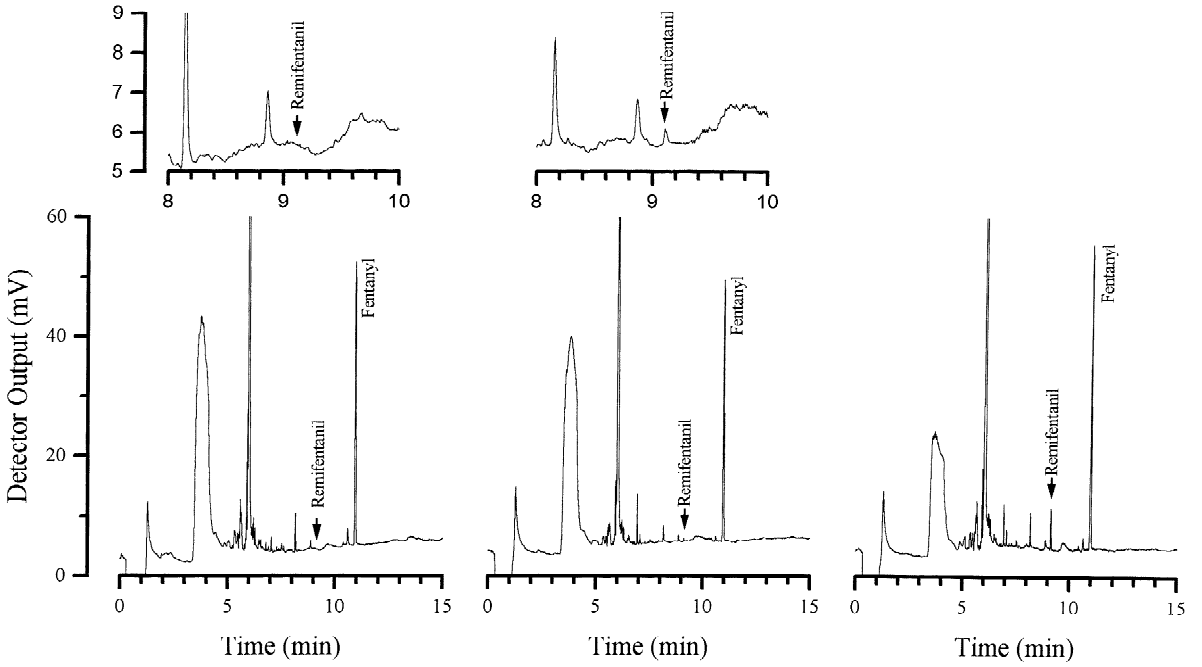

Fig. 2. Representative chromatograms of blank plasma (left panel), patient sample at 0.23 ng/ml (middle panel), and a patient sample (4.45 ng/ml, right panel). Further detail around the remifentanil peak for the blank and the 0.23 ng/ml sample are shown in the inset panels above the main chromatogram in each case.

ever, the mode of operation is somewhat different as the extract is evaporated to dryness while still in the injector port liner before being rapidly vaporised onto the cold (150 °C) analytical column. The solutes of interest were cold-focused before elution at 270 °C. Although we used injection volumes up to 20 μl, corresponding to the entire extract of the sample and a convenient volume to handle in our laboratory, in practice it is possible to use much larger volumes if desired. The programmed temperature vaporiser functions equally well as a standard split injector when extreme sensitivity is not required.

In conclusion, we have developed and validated a robust and convenient assay, using a capillary GC technique with nitrogen-selective detection, suitable for the analysis of remifentanil at all therapeutic concentrations in human blood.

Table 1
Extraction recovery, within- and between-day variations ($n=6$ for each concentration). Mean (% of added concentration) and relative standard deviation (RSD, %)

Remifentanil concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
0.200	81	5.6	105	12	98	14
0.500	82	5.1	98	7.8	103	8.2
2.00	79	4.8	100	7.0	98	6.6
5.00	84	5.3	100	4.4	101	5.6
20.0	83	5.0	100	3.5	103	5.4
100	85	4.8	101	3.8	100	4.5

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