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Analysis of low concentration sufentanil citrate/bupivacaine hydrochloride admixtures, using solid phase extraction followed by high-performance liquid chromatography

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Abstract

A method has been developed for the quantitative determination of low concentrations of sufentanil citrate (1.0 μg/ml), in the presence of bupivacaine hydrochloride (0.125%), in the quality control of pharmaceutical preparations. The main problem in analysis of this combination is the low concentration of sufentanil citrate in the presence of relatively high concentrations of bupivacaine hydrochloride. This paper describes the validation of a HPLC method of sufentanil citrate in an admixture with bupivacaine hydrochloride using solid phase extraction (SPE). The optimized method shows good linearity, precision and accuracy. The limits of detection (0.09 μg/l) and quantification (0.29 μg/l) for sufentanil citrate are lower than the maximal accepted limits. This method is currently used in stability studies.

Keywords: Sufentanil citrate; Bupivacaine hydrochloride; Solid phase extraction; Pharmaceutical admixture; Validation

1. Introduction

Bupivacaine hydrochloride is frequently used in combination with opioids for peridural analgesia. Local anesthetics have been administrated epidurally in combination with opioids to reduce the effective concentration of either drug and thus minimize side effects. Continuous epidural infusion of opioids combined with bupivacaine hydrochloride in postoperative pain relief and during labour is well documented [1,2].

In our hospital, the combination of sufentanil citrate (1.0 μg/ml) with bupivacaine hydrochloride (0.125%) is frequently used epidurally in obstetrics and post-operative pain relief. Sufentanil citrate is a potent synthetic opioid analgesic related to fentanyl. Until now preparation of the admixtures is done by nursing staff immediately before administration using Sufenta Forte® (50 μg/ml) ampoules and Marcaine® vials. From a quality point of view, premixed and autoclaved solutions prepared by the pharmacy should be preferred. A shelf-life investigation is required to stock a premixed solution. Data on the compatibility of sufentanil citrate and bupivacaine hydrochloride after aseptic

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mixing are available [3,4]. Several methods are described for the analysis of sufentanil citrate in combination with bupivacaine hydrochloride but all at least in a fivefold higher concentration of sufentanil citrate. However, no methods have been described separating and analyzing sufentanil citrate in low concentrations in the presence of relatively high concentrations of bupivacaine hydrochloride.

The objective of the present study was to develop a new method which makes it possible to measure low concentrations of sufentanil citrate in presence of large amounts of bupivacaine hydrochloride in a routine pharmaceutical quality control program.

2. Experimental

2.1. Materials and reagents

Sufentanil citrate was supplied as Sufenta Forte® (50 μg/ml) ampoules obtained by Janssen (Beerse, Belgium). Methanol (analytical grade) and acetonitril (HPLC-grade) were purchased from J.T. Baker (Deventer, Netherlands) and were used without further purification. Bupivacaine HCl (Marcaine® 0.25%) was obtained from Astra (Zoetermeer, Netherlands). Both pharmaceutical formulations contained normal saline in isotonic concentrations with no other substances.

2.2. Preparation of standard and sample solutions

Sufenta Forte® (50 μg/ml) ampoules were used as stock solution of sufentanil citrate. A set of standard sufentanil citrate solutions (range: 0.50–1.50 μg/ml) in 0.125% bupivacaine hydrochloride solution were prepared by diluting the sufentanil citrate stock solution with Marcaine® solution 0.25% and deionised water in appropriate amounts. The resulting pharmaceutical formulation prepared in our pharmacy consisted of 1.0 μg/ml sufentanil citrate and 0.125% bupivacaine hydrochloride in 0.9% normal saline. Clomipramine hydrochloride was used as the internal standard at a concentration of 40 mg/l in water.

2.3. Sample collection and solid phase extraction (SPE) of sufentanil citrate

SPE was performed with Sep-Pak cartridges, type vac. 3cc tC-18 (Millipore Waters, Etten-Leur, The Netherlands). The columns were conditioned with 3 ml methanol and 3 ml water. After conditioning, 10 ml sample was spiked with 100 μl internal standard and was slowly poured down the column (flowrate 2–3 ml/min). The column was carefully washed with successively 1 ml deionised water and 3 ml of a phosphate modifier (530 ml deionised water with 300 μl phosphorous acid 85% plus 146 μl TEA, pH = 3.35) and acetonitril (53:47 v/v), and dried under full vacuum for 5 min. The samples were eluted with 3 ml methanol. The volume amounts were tested for optimal extraction efficiency in presence of bupivacaine hydrochloride 0.125% (data not shown). The extracts were dried under nitrogen at 40°C. The residue was dissolved in 100 μl of mobile phase and 50 μl was injected into the HPLC system.

2.4. Instrumentation and chromatographic conditions

All chromatographic analyses were performed on a L-6200 A solvent delivery system, an AS 2000 autosampler and a L 4500 diode-array detector (all Merck, Hitachi, Amsterdam, The Netherlands). Integrations and calculations were carried out using D 7000 HSM software and a Compaq deskpro 466 computer. The detection wavelength was 205 nm and peak area ratios were used for calculations.

The HPLC column was an Inertsil ODS-2 (5 μm) 250 × 4.6 mm (Applied Science, Emmen, The Netherlands). The injection volume was 50 μl and the flow rate of the mobile phase was set at 1.0 ml/min. The analysis of the test solutions took place at ambient temperatures.

The mobile phase used, consisted of an admixture of phosphate modifier and acetonitril (62:38 v/v) and was degassed ultrasonically for 10 min before use. The phosphate modifier was prepared by mixing 620 ml deionised water with

3000 μl phosphorous acid 85%. The pH was set at 3.35.

The stability-indicating capability of the assay, verified by forced degradation of the sufentanil citrate-bupivacaine hydrochloride samples, has been described previously [4,5]. Bupivacaine hydrochloride is stable in the pH range from 4–6.5 and no degradation products were detected in other stability studies [6].

2.5. Validation

This paper describes the validation of a chromatographic method for the determination of sufentanil citrate based on fractionated solid phase extraction according to recent guidelines of The European Agency for the Evaluation of Medicinal Products (EMEA) [7].

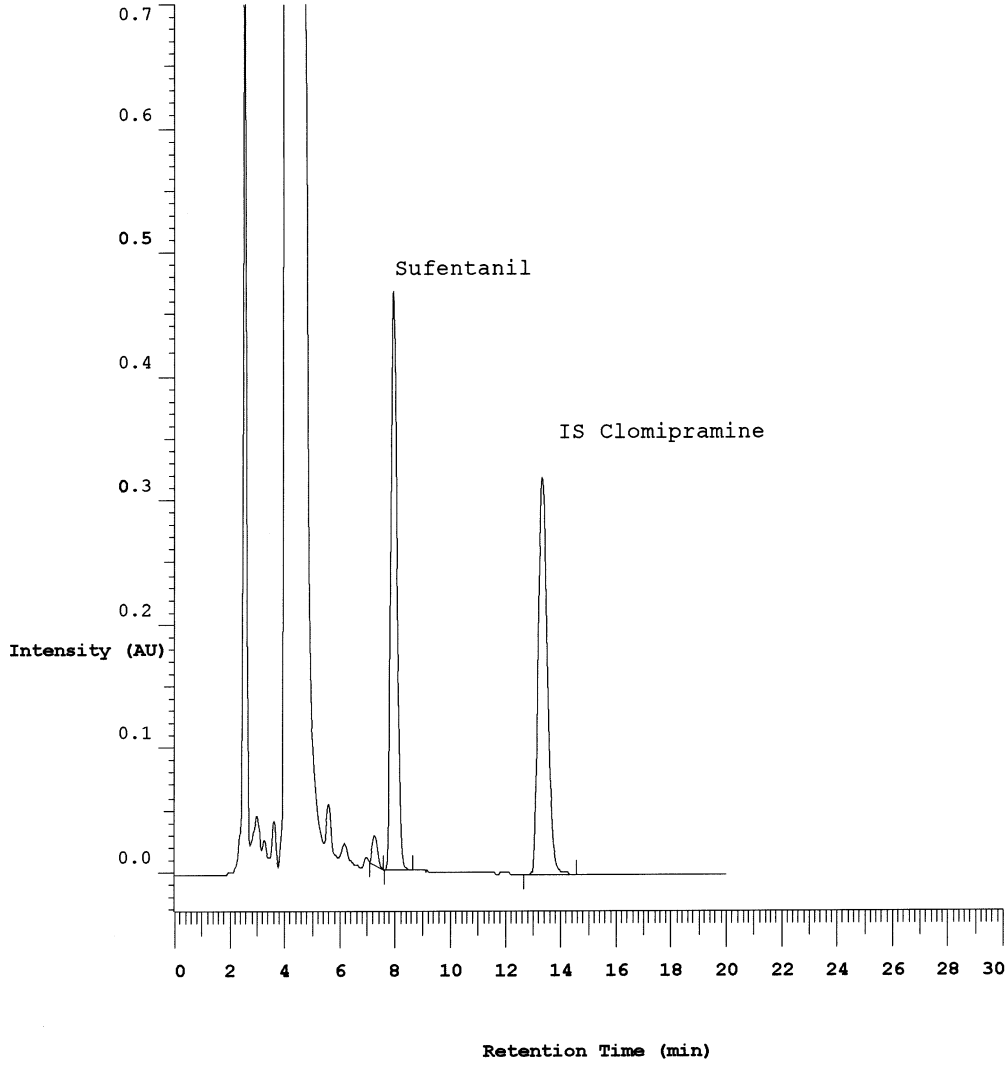

Fig. 1. Representative HPLC chromatogram of sufentanil citrate (1.0 μg/ml) and clomipramine as internal standard in presence of bupivacaine hydrochloride after SPE. Chromatographic conditions: 5 μm Inertsil ODS-2 (250 × 4,6 mm) column with phosphate modifier (620 ml deionised water with 3000 μl phosphorous acid 85% (pH 3.35))-acetonitril (62:38 v/v) at a flow-rate of 1.0 ml/min. UV detection at 205 nm. (AU is absorbent unit, min is minutes).

Table 1
Validation parameters for sufentanil citrate 1.0 μg/ml in bupivacaine hydrochloride 0.125% ($n = 6$)

Extraction efficiency (sufentanil citrate) [mean ± vc]	87.5% ± 3.7%
Extraction efficiency (internal standard) [mean ± vc]	81.8% ± 9.1%
Precision (within run) [mean ± vc]	1.0 μg/ml ± 4.2%
Precision (between run) [mean ± vc]	1.0 μg/ml ± 11.4%
Accuracy	1.0 μg/ml [105.8%]
Limit of detection (LOD)	0.09 μg/ml
Limit of quantification (LOQ)	0.29 μg/ml

Linearity, precision and accuracy were determined on aqueous samples. The linearity of the method for sufentanil citrate was confirmed, using statistical tests (goodness of fit and lack of fit) on five calibration standards covering the range 50–150% of the target concentration. Each standard solution was assayed twice and a standard curve of the sufentanil citrate-internal standard peak area ratios versus concentration was generated using linear regression.

3. Results

In this study we used HPLC coupled with UV detection to quantificate low amounts of sufentanil citrate in the presence of bupivacaine hydrochloride. The selectivity of the procedure was determined by resolution calculation (claim > 1.5). Fig. 1 shows an example of a chromatogram obtained from a calibration sample spiked with internal standard. The selectivity of the procedure was determined by the resolution of the peaks in the HPLC chromatograms. The resolution of the peaks was > 1.5, and was considered well.

3.1. Extraction efficiency

The recovery of sufentanil citrate was 78.5 ± 3.7% estimated at a concentration of 1.0 μg/ml ($n = 6$). The recovery of clomipramine HCl was 81.8 ± 9.1% ($n = 6$).

3.2. Linearity

For sufentanil citrate we verified the linearity of the method by injecting five concentrations in duplo between 50 and 150% of the expected concentration. In this case, we reported the chromatographic peak area ratios of the drug-internal standard as a function of its concentration. The linearity was tested and demonstrated over the range of 50–150% with a goodness-of-fit and lack-of-fit test.

3.3. Precision and repeatability

The day-to-day precision was estimated for a fixed concentration of 1.0 μg/ml sufentanil citrate. The samples were measures over a range of a couple of weeks. The coefficient of variation for sufentanil citrate was 11.4% ($n = 6$). The within-run precision is assessed by 6 determinations at 100% of the test concentration (1.0 μg/ml). The coefficient of variation was 4.2% (all sufentanil citrate). As shown in Table 1, the day to day and without-run precision expressed by the relative standard deviation is acceptable.

3.4. Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ), obtained for signal-to-noise ratios of 3:1 and 10:1, respectively, are shown in Table 1. For sufentanil citrate the limit of quantification correspond to 0.29 μg/ml of sufentanil citrate. These values are clearly lower than required for stability studies.

4. Discussion

With the described method we are able to determine sufentanil citrate in a concentration of 1.0 μg/ml in presence of relatively high concentrations bupivacaine hydrochloride. This will be advantageous over methods described in literature in which concentrations of 5 μg/ml sufentanil citrate or more are measured in combination the bupivacaine hydrochloride [3,4]. Recent studies show that normally clinically used concentrations of

sufentanil citrate in admixture with bupivacaine hydrochloride are within the range of 0.5 μg/ml to 2 μg/ml.

An interesting phenomenon is the fact that the extraction efficiency of sufentanil citrate is negligible using a sufentanil citrate solution without the presence bupivacaine hydrochloride. Fig. 2 shows a chromatogram of sufentanil citrate and internal standard after SPE without bupivacine hydrochloride present in the admixture. Loss of sufentanil citrate by sorption on the materials used for preparation is a know phenomenon [8,9]. Using a combination of sufentanil citrate with bupivacaine hydrochloride reduces the adsorption of sufentanil citrate into the PVC wall of a container in comparison to sufentanil citrate alone. Sorption of a drug by a synthetic polymer is dependent on a number of different factors like

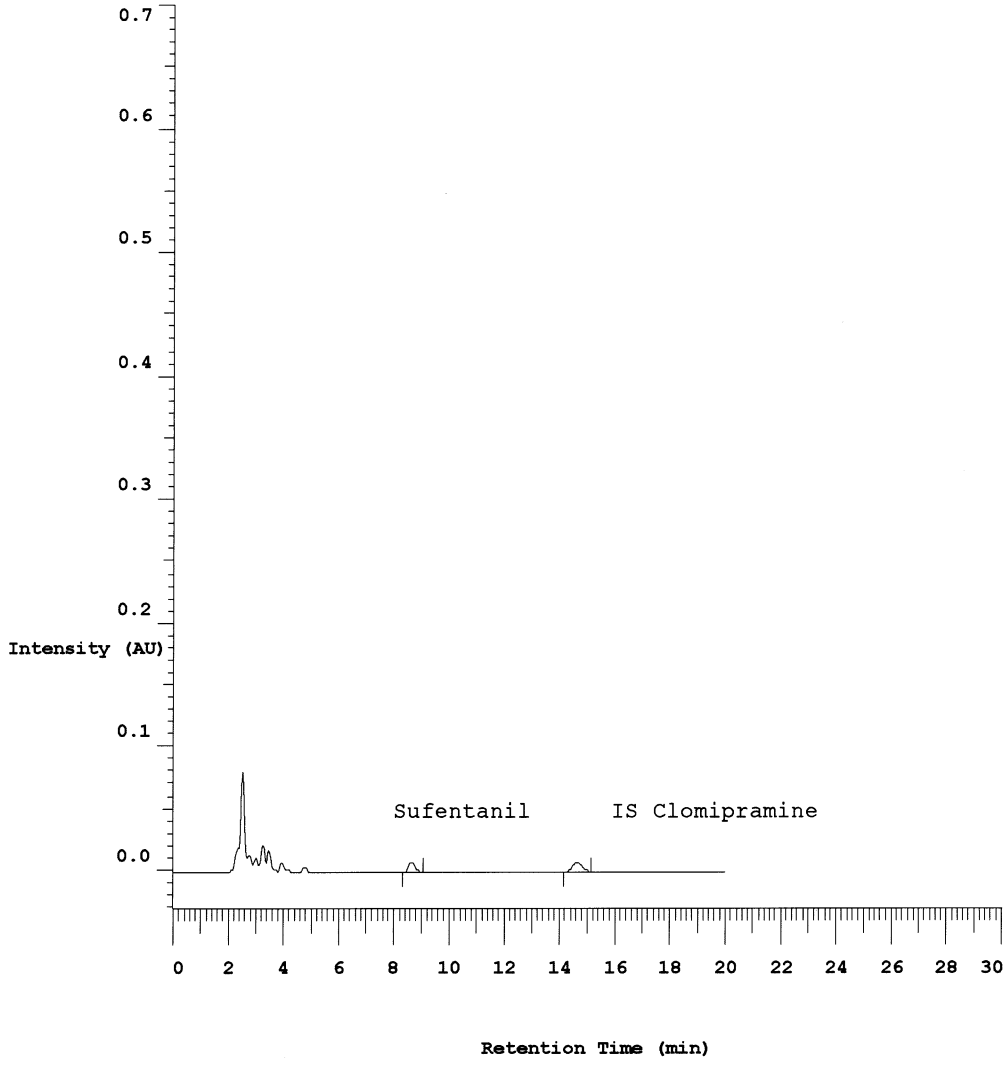

Fig. 2. Representative HPLC chromatogram of sufentanil citrate (1.0 mg/ml) and clomipramine as internal standard in absence of bupivacaine hydrochloride after SPE. Chromatographic conditions: 5 μm Inertsil ODS-2 (250 × 4,6 mm) column with phosphate modifier (620 ml deionised water with 3000 μl phosphorous acid 85% (pH 3.35))-acetonitril (62:38 v/v) at a flow-rate of 1.0 ml/min. UV detection at 205 nm. (AU is absorbent unit, min is minutes).

the surface:volume ratio and the physicochemical characteristics of the solute and the polymer, respectively [10,11].

We have demonstrated that SPE gave good results for the separation of low amount sufentanil citrate in presence of huge amount of bupivacaine hydrochloride. The precision, accuracy and linearity for the bupivacaine hydrochloride assay were satisfactory (data not shown).

Our method is sensitive enough to use in pharmaceutical quality control. Work is in progress to study the compatibility and behavior of both drugs, in the described concentrations, after autoclaving.

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