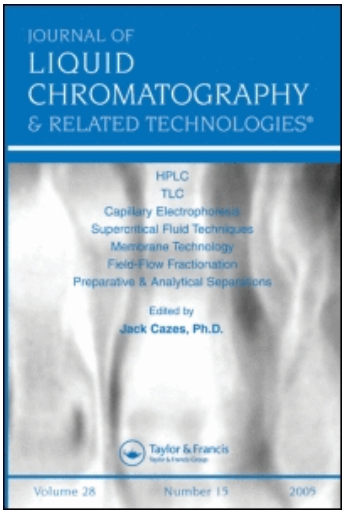

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HPLC DETERMINATION OF A PROPOFOL AND REMIFENTANIL MIXTURE

Karthick Vishwanathan[a]; James T. Stewart[a]
[a] Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA, U.S.A.

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HPLC DETERMINATION OF A PROPOFOL AND REMIFENTANIL MIXTURE

Karthick Vishwanathan, James T. Stewart

Department of Pharmaceutical and Biomedical Sciences
College of Pharmacy
The University of Georgia
Athens, GA 30602-2352, USA

ABSTRACT

Two separate High Performance Liquid Chromatography procedures have been developed for the assay of a remifentanil - propofol mixture. The separation and quantitation of remifentanil were achieved on an ethylsilane column at ambient temperature (23°C) using a mobile phase of 62:38 v/v 0.01 M phosphate buffer pH 2.5-acetonitrile at a flow rate of 2 mL/min with detection at 210 nm. A methylsilane column at 23°C using the same mobile phase at a flow rate of 1.0mL/min with detection at 210 nm was used for propofol. The methods showed linearity for remifentanil and propofol in the 1.0-45 and 15-75 μg/mL ranges, respectively. Accuracy and precision were in 0.08 - 2.3% and 0.2 - 1.2% ranges, respectively, for both drugs. The limits of detection for remifentanil and propofol were 200 and 250 ng/mL, respectively, based on a signal to noise ratio of 3 and a 10 μL injection.

INTRODUCTION

A mixture of propofol(10mg/mL) and remifentanil(5 and 50μg/mL) can be administered as a perioperative injection in U.S. hospitals. Interest in our laboratories in the stability and compatibility of the drug mixture over time in polyvinyl chloride (PVC) bags and polypropylene syringes required the development of an HPLC method. A search of the literature indicated that an HPLC method was not available to assay for the mixture concurrently in a single injection.

Propofol has been analyzed primarily by gas chromatographic methods.[1-3] The assays are based on liquid-liquid extraction clean-up procedures and were used for determining drug levels in plasma. An HPTLC assay was reported to determine the sorption of propofol in infusion containers.[4] A comparison of HPLC to second derivative UV spectroscopy was reported for a propofol oil-in-water emulsion dosage form.[5] Other HPLC procedures were based on reverse phase chromatography and used UV, electrochemical, and fluorescence detection to determine propofol levels in serum or plasma samples.[6-8]

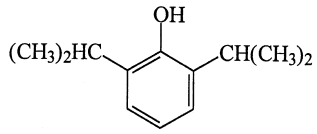
Remifentanil is structurally similar to fentanyl. A number of analytical methods have been developed to quantitate fentanyl and related agents in biological matrices including HPLC,[9,10] radioimmunoassays,[11] radioreceptor[12] as well as gas chromatography-mass spectrometry methods.[13,14] To evaluate pharmacokinetics, GC-HRMS-SIM[15] and HPLC-UV methods[16,17] have been developed to quantitate remifentanil in human, dog and rat blood samples.

Since the concentration of propofol in an actual injection mixture can be 200-2000 times that of remifentanil, a single isocratic method that will quantitatively analyze both drugs simultaneously was impossible to develop. Thus, separate isocratic HPLC methods were used to analyze each drug in the mixture. Separations were achieved within 12 min. on each system at ambient temperature with sensitivity in the ng/mL range.

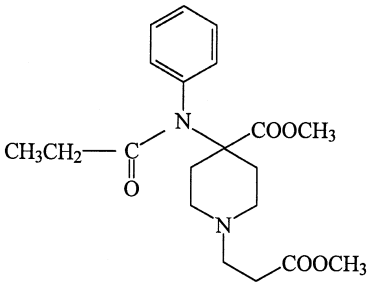
EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the compounds studied are shown in Figure 1. Remifentanil Hydrochloride working standard (Lot G187084B) was secured from Glaxo Wellcome, Inc., (Research Triangle Park, NC 27709). Propofol working standard (Lot adm-710135/91A) was obtained from Zeneca Pharmaceuticals (Wilmington DE, 19850 - 5437). Acetonitrile (J. T. Baker,



Propofol



Remifentanil

Figure 1. Structural formulae of medications studied.

Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076). Monobasic ammonium phosphate, phosphoric acid and hydrochloric acid were Baker analyzed reagents.

INSTRUMENTATION

The chromatographic separations were performed on two HPLC systems. System 1 consisted of a Beckman Model 110B solvent delivery module (San Ramon, CA 94583), an Alcott Model 728 auto-sampler (Norcross, GA 30093) equipped with a 10μL loop, a Varian variable wavelength UV detector (Walnut Creek, CA 94598), a Shimadzu Model C-R3A integrator (Columbia, MD 21046). Separation was accomplished on an ethylsilane (220 x 4.6 mm i.d., 10μm particle size, Brownlee Spheri 10-RP-2, Applied Biosystems, Inc., San Jose, CA 94134) column. System 2 consisted of a Beckman Model 110B solvent delivery Module (San Ramon, CA 94583), an Alcott Model 738 auto-

sampler (Norcross, GA 30093) equipped with a 10μL loop, a Waters model 481 LC spectrophotometer (Milford, MA 01757), a Hewlett Packard HP3394A integrator (Avondale, PA 19311). Separation was achieved on a methylsilane (250 x 4.6mm i.d., 5 μm particle size, Phenomenex IB SIL C1, Torrance, CA 90501) column. The mobile phase for both analytes consisted of 62:38 v/v 0.01M aqueous monobasic ammonium phosphate pH 2.5 (adjusted with 1N phosphoric acid)-acetonitrile. The mobile phases were filtered through a 0.45 μm Nylon-66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use. The flow rate was 2mL/min for remifentanil and 1mL/min for propofol with each detector set at 210 nm.

Preparation of Standard Solutions

A 1 mg/mL solution of remifentanil base was prepared by accurately weighing 1.11 mg of remifentanil hydrochloride (equivalent to 1 mg of the free base) and dissolving it in 1 mL of sterile water (Stock A). Propofol (100 mg) was weighed and dissolved in 10 mL of acetonitrile to give a 10 mg/mL solution (Stock B). Aliquots of Stock A were diluted with 0.01M hydrochloric acid to give standard solutions containing 5,10, 20, 30, 40, and 45 μg/mL of remifentanil. Accurately measured portions of Stock B were also diluted with 0.01M hydrochloric acid to provide standard solutions containing 20, 30, 40, 50, 60, and 70 μg/mL of propofol. These standard solutions were used to construct six point calibration curves for each analyte in its respective HPLC system. Spiked mixtures containing A: 5 μg/mL and 10 mg/mL of remifentanil and propofol, respectively, and B: 50 μg/mL and 10 mg/mL of remifentanil and propofol, respectively, were prepared and used to determine accuracy and precision for each analyte using the HPLC methods. Quantitation was based on linear regression analysis of analyte peak height for remifentanil and peak area for propofol versus analyte concentration in μg/mL.

RESULTS AND DISCUSSION

There were no reports in the scientific literature describing the separation and quantitation of 5 and 50μg/mL remifentanil with 10mg/mL propofol in a single injection mixture. Initial studies to develop a single isocratic HPLC method for the two analytes involved the use of underivatized silica, phenyl, octyl, and octadecylsilane columns with various mobile phases containing acetonitrile or methanol 0.01 M -aqueous phosphate buffers pH 2.5 at 1-2 mL/min. The separation of both drugs using a single HPLC system was possible (see Figure 2, III), but since the concentration of propofol in the actual injection mixture was 200-2000 times the concentration of remifentanil, the linear concentration range of each drug for quantitation purposes was entirely different

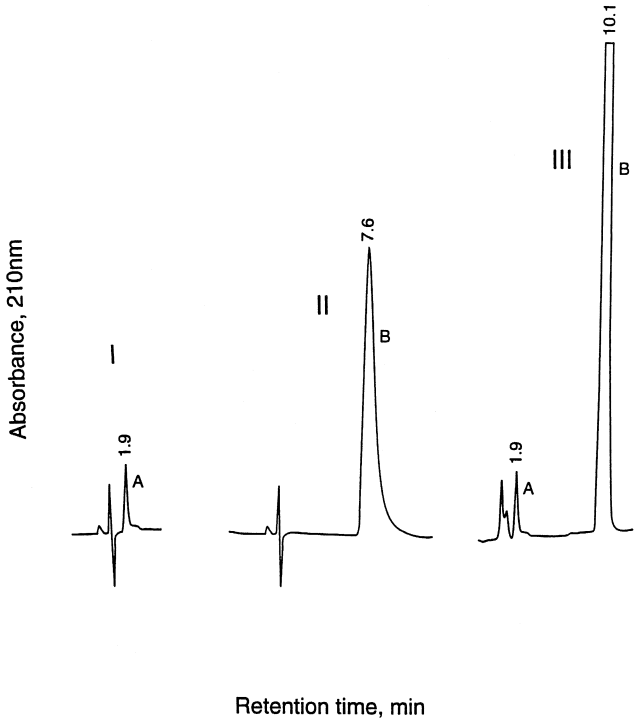


Figure 2. (1) A typical chromatogram of (A) Remifentanil (5 μg/mL) on an ethylsilane column at ambient temperature using a mobile phase of 62:38 v/v 0.01M phosphate buffer pH 2.5 - acetonitrile at a 2 mL/min flow rate at 210 nm. (II) A typical chromatogram of (B) propofol (50 μg/mL) on a methylsilane column at ambient temperature using a mobile phase of 62:38 v/v 00.1 M phosphate buffer pH 2.5-acetonitrile at a 1 mL/min flow rate at 210 nm. (III) A typical chromatogram of Mixture A containing remifentanil (5μg/mL)-propofol (10 mg/mL) injected on an ethylsilane column at ambient temperature using a mobile phase of 62:38 v/v 0.01 M phosphate buffer pH 2.5 - acetonitrile at a 2 mL/min flow rate at 210 nm.

(1-45 μg/mL for remifentanyl and 15-75 μg/mL for propofol). This necessitated the preparation of separate calibration curves for each analyte and involved appropriate dilutions of an actual injection mixture to fit within the respective linear ranges. The use of different detection sensitivities for both analytes on the same columns also did not allow quantifiable data. Thus, two separate HPLC systems were used for the analysis of the mixture. The best resolution for remifentanil was obtained on an ethylsilane column using a 62:38 v/v phosphate buffer pH 2.5-acetonitrile mobile phase with a total run time of 12 min. and that of propofol was obtained on a methylsilane column using the same mobile phase

Table 1

Analytical Figures of Merit for Remifentanil and Propofol

Analyte	HPLC System	r^{2a}	System Suitability[b]	LOD[c] ng/mL	K'	Theoretical Plates[d]	Tailing Factor[e]
Remifentanil	1	0.9997	0.98	200	0.73	346	1.1
Propofol	2	0.9994	1.43	250	2.26	1628	1.3

[a] Range examined from 1-45 μg/mL remifentanil (n=9) and 15-75 μg/mL propofol (n=9). Mobile phase consisted of 62.38 v/v 0.01M phosphate buffer, pH 2.5-Acetonitrile, and detection at 210nm.

[b] Mean RSD % of 6 replicate injections at 20 μg/mL remifentanil and 25 μg/mL propofol at 210 nm.

[c] Limit of detection, S/N =3.

[d] Calculated as $N=16\ (tr/w)^2$.

[e] Calculated as 10% peak height.

with a total run time of 10 min. It had been previously determined that any propofol or remifentanil present in the analytical samples will have eluted from the column prior to injection of another sample. Typical chromatograms showing the separation of the analytes are shown in Fig. 2.

The λ max for propofol is 263 nm and remifentanil absorbs strongly at 210 nm. The latter wavelength was chosen for this study based upon the desired detection of remifentanil at low concentrations in the mixture compared to propofol. The use of 210 nm provided good accuracy and precision data for each analyte.

The HPLC methods showed concentration versus absorbance linearity for remifentanil and propofol in the 1 - 45 μg/mL and 15 - 75 μg/mL ranges, respectively, at 210 nm. Table 1 shows the analytical figures of merit for each analyte in each system. The drugs were acid and base degraded using 0.1N hydrochloric acid and 0.1N sodium hydroxide, respectively, and the principle drug peaks were verified using a photodiode array detector (Model 990, Waters Associates, Milford, MA 01757) to show that none of the degradation products of each analyte interfered with the quantitation of the other drug at 210 nm.

These experiments were performed on solutions of remifentanil in acid and base for 5 min followed by neutralization. For propofol, the drug solutions in acid and base were heated at 85^0C for 24 hrs prior to neutralization.

Table 2

Accuracy and Precision Using Spiked Drug Mixtures A and B

Mixture	Concn. Added	Concn. Found[a]	Percent Error	RSD (%)
A				
Remifentanil	5 μg/mL	5.09±0.08 μg/mL	1.8	1.6
Propofol	10 mg/mL	10.4±0.12 μg/mL	0.44	1.2
B				
Remifentanil	50 μg/mL	49.78±0.40 μg/mL	0.44	0.3
Propofol	10 mg/mL	9.94±0.16 mg/mL	0.60	1.6

[a] Mean ± Standard deviation based on n=3.

Percent error and precision of the methods were evaluated using two spiked mixtures of analytes. The results shown in Table 2 indicate that the procedure gives acceptable accuracy and precision for each analyte. Intra-day variabilities of each assay for remifentanil and propofol expressed as % RSD were 0.98 and 1.43% (n=6), respectively.

Inter-day variability of each assay of these drugs were 1.2 and 1.5% (n=18 over 3 days), respectively.

In summary, two HPLC systems using either a methylsilane or ethylsilane column and an aqueous 0.01 M pH 2.5 buffer-acetonitrile mobile phase were shown to be amenable for the separation and quantitation of a remifentanil - propofol mixture. Both methylsilane and ethylsilane stationary phases were found to be very stable over a 4 month analytical development period with propofol and remifentanil, respectively. There were no observed significant changes in retention time and peak tailing.

This study suggests that these HPLC methods could be used to investigate the chemical stability of a mixture of these drugs in PVC bags and polypropylene syringes.

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