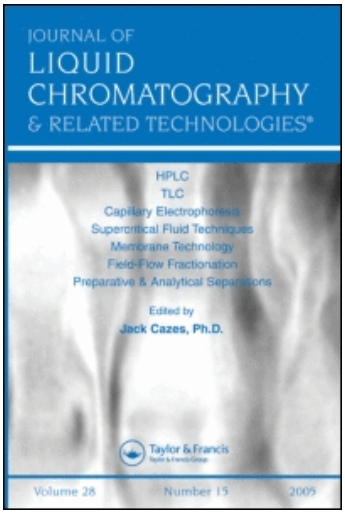

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HPLC Determination of Propofol-Thiopental Sodium and Propofol-Ondansetron Mixtures

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HPLC DETERMINATION OF PROPOFOL-THIOPENTAL SODIUM AND PROPOFOL-ONDANSETRON MIXTURES

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ABSTRACT

High performance liquid chromatography procedures have been developed for the assay of propofol-thiopental sodium and propofol-ondansetron mixtures. The separation and quantitation of propofol-thiopental sodium were performed on a stable bond phenyl column at ambient temperature using a mobile phase of 55:45 v/v aqueous 0.01 M monobasic postassium phosphate pH 4 - acetonitrile at a flow rate of 1 mL/min, with detection set at 235 nm. The separation was achieved within 20 min. Propofol and thiopental sodium were linear in the 12.7 - 38 and 31.4 - 94 μg/mL ranges, respectively. Accuracy and precision were in the range 0.2 - 2.6 and 0.2 - 3.2%, respectively, for the two analytes and the limits of detection for propofol and thiopental sodium were 1210 and 317 ng/mL, respectively, based on a signal to noise ratio of 2 and a 20 μL injection. The separation and quantitation of the propofol-ondansetron mixture was achieved on a 10 μm particle size phenyl column.,using a mobile phase of 50:50 v/v aqueous 0.01 M monobasic potassium phosphate pH 4 - acetonitrile at a flow rate of 1 mL/min, with detection set at 268 nm. The separation was achieved within 15 min. Propofol and

ondansetron were linear in the 2.5 - 37.5 and 0.48 - 7.2 μg/mL ranges, respectively. Accuracy and precision were in the range 0.4 - 2,4 and 0.2 - 0.6%, respectively, for the two analytes and the limits of detection for propofol and ondansetron were 117 and 61 ng/mL, respectively, based on a signal to noise ratio of 2 and a 20 μL injection.

INTRODUCTION

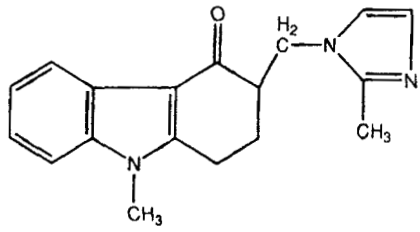
Mixtures of propofol-thiopental sodium (Mixture A) and propofol-ondansetron (Mixture B) are administered as perioperative injections in U.S. hospitals. Interest in this laboratory, in the stability and compatibility of each drug mixture over time, required the development of HPLC methods. A search of the literature indicated that HPLC methods were not available to assay each analyte in Mixture A or Mixture B, 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]

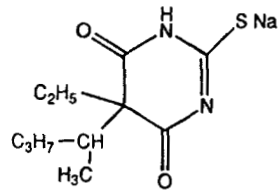
Thiopental sodium has been analyzed by a variety of methods. The official USP 23 method is based on UV spectrophotometry at 304 nm.[9] Other procedures utilize stripping voltammetry, HPLC, GC and micellar electrokinetic chromatography using chemically modified cyclodextrins, to determine thiopental sodium in a myriad of samples.[10-14]

Ondansetron has been assayed by high performance thin layer chromatography (HPTLC), HPLC methods and radioimmunoassay methods. The HPTLC method was developed especially for plasma samples, but the sample output was low and the equipment is not generally available in most laboratories.[15] The HPLC assays used either a silica column with an aqueous-organic mobile phase, or a cyanopropyl column operated in the reverse phase mode.[16-17]

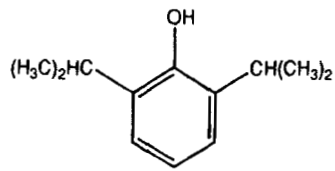
Detection of the analyte was either by UV at 305 nm, or radiochemical detection. The radioimmunoassay was combined with sample cleanup, using a cyanopropyl solid phase extraction cartridge to provide a subnanogram per mL determination of ondansetron.[18]



ONDANSETRON



THIOPENTAL SODIUM



PROPOFOL

Figure 1. Chemical Structures of Compounds Studied.

In this paper, isocratic HPLC assays are presented for the simultaneous analysis of propofol and thiopental sodium (Mixture A), and propofol and ondansetron (Mixture B) mixtures. Both mixtures were separated on phenyl columns using aqueous phosphate buffer pH 4 - acetonitrile eluents. Each separation was achieved within 15-20 min with sensitivity generally in the lower ng to lower μg/mL range for all 3 analytes.

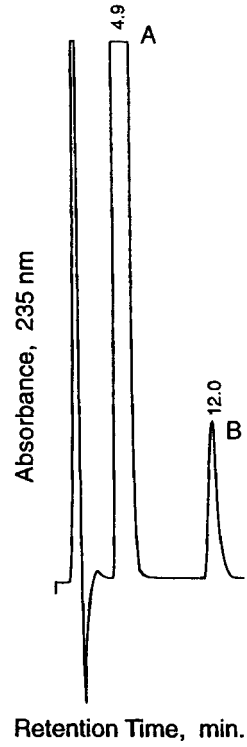


Figure 2 Typical HPLC Chromatogram of thiopental sodium (A) and propofol (B) on a phenyl column with 55:45 v/v 0.01 M phosphate buffer pH 4 - acetonitrile. See Experimental Section for assay conditions.

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the compounds studied are shown in Figure 1. Thiopental Sodium was a gift from Abbott Laboratories (North Chicago, IL) and propofol was obtained from Stuart Pharmaceuticals (Wilmington, DE 19897). Ondansetron hydrochloride (Lot AWS 332A) was a gift from Glaxo, Inc. (Research Triangle Park, NC 27709). Acetonitrile (J.T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, Ga 30076). Monobasic potassium phosphate and concentrated phosphoric acid were Baker analyzed reagents.

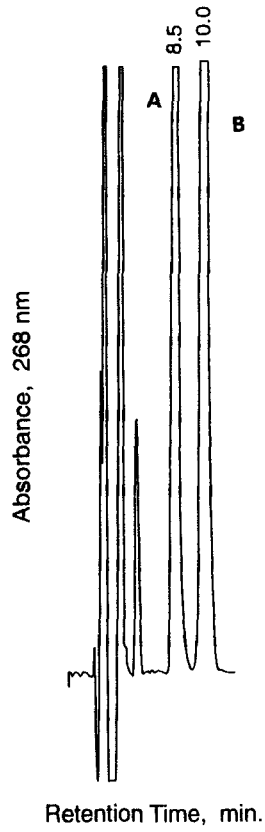


Figure 3 Typical HPLC Chromatogram of ondansetron (A) and propofol (B) on a phenyl column with 50:50 v/v 0.01 M phosphate buffer pH 4 - acetonitrile. See Experimental Section for assay conditions.

Instrumentation

The chromatographic separations were performed on an HPLC system consisting of a Waters Model 501 pump (Milford, MA 01757), an Alcott Model 728 autosampler (Norcross, GA 30093) equipped with a 20 µL loop, a Beckman Model 163 variable wavelength UV-VIS detector (Fullerton, CA 92634) and a Hewlett-Packard Model 3395 integrator (Avondale, PA 19311). Separation of Mixture A was achieved on a 150 mm phenyl column (Zorbax SB, 4.6 mm i.d., 5 µm particle size, MacMod Analytical, Chadds Ford, PA 19317). The mobile phase consisted of 55:45 v/v 0.01 M aqueous monobasic potassium phosphate

pH 4.0 (adjusted with 10% phosphoric acid)-acetonitrile. The separation of Mixture B was accomplished on a 300 mm phenyl column (T-Bondapak, 4.6 mm i.d., 10 μm particle size, Waters, Milford, MA 01757). The mobile phase consisted of 50:50 v/v 0.01 M aqueous monobasic potassium phosphate pH 4.0 (adjusted with 10% 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 set at 1.0 mL/min for both mixtures and the detector was set at 235 nm for Mixture A and 268 nm for Mixture B.

Preparation of Standard Solutions

A combined standard solution containing propofol and thiopental sodium was prepared by accurately weighing 0.38 mg propofol and 1.03 mg thiopental sodium in a 10 mL volumetric flask. Another standard solution containing propofol and ondansetron was prepared by accurately weighing 0.38 mg propofol and 0.083 mg ondansetron hydrochloride in a 10 mL volumetric flask. Methanol was added to each mixture and the flasks were shaken vigorously for 2 min, followed by methanol to volume. Dilutions of the combined propofol-thiopental sodium and propofol-ondansetron standard solutions gave solutions in the 12.7 - 38 μg/mL and 31.4 - 94 μg/mL range for propofol-thiopental sodium and 2.5 - 37.5 μg/mL and 0.48 - 7.2 μg/mL range for propofol-ondansetron, respectively. Additional dilutions of Mixture A and Mixture B standard solution were prepared in methanol, to serve as spiked samples for each analyte to determine accuracy and precision. Quantitation was based on linear regression analyis of analyte peak height versus analyte concentration in μg/mL.

RESULTS AND DISCUSSION

There were no reports in the literature describing separations of propofol-thiopental sodium and propofol-ondansetron mixtures. Initial studies to develop HPLC methods for each mixture using isocratic conditions, involved the use of underivatized silica, phenyl, octyl, deactivated octyl and octadecyl columns, with various mobile phases containing methanol-aqueous phosphate buffers and/or acetonitrile-aqueous phosphate buffer at 1 mL/min. The best resolution of the analytes in Mixtures A was obtained on a 150 mm stable bond 5 μm phenyl column, using 55:45 v/v phosphate buffer pH 4 - acetonitrile. Mixture B was best resolved on a 300 mm 10 μm phenyl column using 50:50 v/v phosphate buffer pH 4 - acetonitrile.

Table 1

Analytical Figures of Merit for Propofol-Thiopental Sodium and Propofol-Ondansetron Mixtures

Mixture	r^{2a}	System Suitability	LOD[c] ng/mL	k	Theoretical Plates[d]	Tailing Factor	Rs
A							
Propofol	0.9996	1.43	1210	6.6	1039	1.1	6.0
Thiopental Sodium	0.9999	1.13	317	2.1	482	1.1	
B							
Propofol	0.9999	0.48	117	2.8	2755	1.1	4.0
Ondansetron	0.9999	0.65	61	1.7	1322	1.3	

[a] Range examined from 12.7 - 38 µg/mL propofol (n=6) and 31.4 - 94 µg/mL thiopental sodium for Mixture A at 235 nm and 2.5 - 37.5 µg/mL propofol and 0.48 - 7.2 µg/mL ondansetron for Mixture B at 268 nm.
[b] Mean RSD% of 6 replicate injections at 25.3 µg/mL propofol and 62.7 µg/mL thiopental sodium for Mixture A at 235 nm and 25 µg/mL propofol and 4.8 µg/mL ondansetron for Mixture B at 268 nm.
[c] Limit of Detection, S/N = 2.
[d] Calculated at N=16 $(tr/w)^2$.
[e] calculated at 5% peak height

The columns also allowed the separation of methylparaben (preservative found in most commercial injections) from the analytes. Typical chromatograms showing the separation of each mixture are shown in Figures 2 and 3.

From an earlier study in our lab, it was shown that propofol and thiopental sodium absorb strongly at 235 nm in an acetonitrile - phosphate buffer system. It was also determined, that propofol and ondansetron absorb around 268 nm in essentially an identical mobile phase. Therefore, 235 and 268 nm were selected as the detection wavelengths for Mixture A and B, respectively, since they provided good accuracy and precision data for the two component mixtures.

Table 2

Accuracy and Precision Using Samples With Added Drug

Mixture	Concn Added μg/mL	Conc Found μg/mL[a]	Percent Error	RSD%
A				
Propofol	16.55	16.12 ± 0.51	2.6	3.2
	29.72	29.47± 0.39	1.1	1.3
Thiopental	43.34	43.72 ± 0.18	0.9	0.4
Sodium	78.01	77.24 ± 0.13	1.0	0.2
B				
Propofol	12.10	12.05 ± 0.03	0.4	0.3
	29.10	29.05 ± 0.10	0.2	0.3
Ondansetron	2.40	2.35 ± 0.02	2.1	0.9
	5.76	5.62 ± 0.01	2.4	0.2

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

The HPLC method for Mixture A showed concentration versus absorbance linearity for propofol-thiopental sodium in the 12.7 - 38 μg/mL and 31.4 - 94 μg/mL ranges, respectively, at 235 nm. Table 1 gives the analytical figures of merit for each of the analytes in Mixture A. The HPLC method for Mixture B showed concentration versus absorbance linearity for propofol-ondansetron in the 2.5 - 37.5 μg/mL and 0.48 - 7.2 μg/mL ranges, respectively, at 268nm. Table 1 also gives the analytical figures of merit for each of the analytes in Mixture B.

A photodiode array detector (Model 990, Waters Associates, Milford, MA 01757) was used to verify that none of the degradation products of the analytes, in either Mixture A or B (analyzed under their respective analytical conditions), interfered with the quantitation of each drug at 235 or 268 nm.

These experiments were performed on solutions of each drug, in 0.9% sodium chloride injection after they has been degraded for 1-6 hr at ambient temperature and 45-60°C with 0.1 N hydrochloric acid, 0.05 N sodium hydroxide, and 3-30% hydrogen peroxide solutions.

Percent error and precision of the methods were evaluated using spiked samples containing each analyte. The results for mixtures A and B are shown in Table 2. The results indicate, that the procedures give acceptable accuracy and precision for the analytes in both mixtures.

Intraday variabilities for propofol-thiopental sodium (Mixture A) expressed as % RSD, were 1.13 and 1.43% (n=6), respectively. Interday variabilities of the assay for propofol and thiopental sodium were 0.82 and 0.66% (n=18 over 3 days), respectively. Intraday variabilities for propofol-ondansetron (Mixture B) expressed as % RSD, were 0.48 and 0.65% (n=6), respectively. Interday variabilities of the assay were 0.36 and 0.59% (n=18 over 3 days) for propofol and ondansetron, respectively.

In summary, a 5 μm stable bond phenyl column and a 10 μm phenyl column with aqueous phosphate buffer pH 4-acetonitrile mobile phases, were shown to be suitable for the separation and quantitation of a propofol-thiopental sodium mixture (A) and a propofol-ondansetron mixture (B). This study suggests that the HPLC methods developed, herein, can be used to investigate the chemical stability of these analyte mixtures.

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