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# HPLC determination of cisatracurium besylate and propofol mixtures with LC-MS identification of degradation products[1]

H. Zhang, P. Wang, M.G. Bartlett, J.T. Stewart *

*Department of Medicinal Chemistry, College of Pharmacy, University of Georgia, Athens, GA 30602–2352, USA*

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**Abstract**

A stability-indicating HPLC method was developed to simultaneously determine cisatracurium besylate and propofol in mixtures. The effects of organic modifier, ionic strength and the pH of the mobile phase on resolution and retention were investigated. A baseline separation was achieved on an octadecylsilane column with an isocratic mobile phase of acetonitrile–ammonium formate (pH 5.2; 0.3 M) (50:50, v/v). Cisatracurium and propofol were confirmed by both retention time and mass-to-charge ratio using LC-MS. The degradation products of cisatracurium were identified by ESI positive-ion detection as Hofmann elimination and ester hydrolysis products of cisatracurium. There were no propofol degradation products observed. The quantitation of the two drugs was accomplished using UV detection at 280 nm. This method showed linearity for cisatracurium besylate and propofol in the 8–128 and 37–592 μg $ml^{-1}$ ranges, respectively. Accuracy and precision were in the 0.4–1.4 and 0.4–2.9% ranges respectively, for both analytes. Published by Elsevier Science B.V. All rights reserved.

*Keywords:* HPLC; LC-MS; Determination; Cisatracurium; Propofol; Degradation

## 1. Introduction

Cisatracurium besylate is one of the ten stereoisomers of atracurium besylate and constitutes approximately 15% of this mixture. It is a non-depolarizing, intermediate-acting neuromuscular blocking agent used for skeletal muscle relaxation during surgery or mechanical ventilation [1]. The neuromuscular blocking potency of cisatracurium is approximately 3-fold that of atracurium besylate.

Propofol, 2,6-diisopropylphenol, exists as an oil at ambient temperature. The drug is an intravenous hypnotic agent used for both induction and maintenance of anaesthesia. It is also useful for sedation as a supplement to regional anaesthetics and in critically ill patients confined to intensive care units (ICU) [1]. Propofol is only slightly soluble in water, thus necessitating its formulation as an oil-in-water emulsion.

A mixture of cisatracurium and propofol injections is administered as a perioperative injection

* Corresponding author. Tel.: +1 706 5425770; fax: +1 706 5425358; e-mail: jstewart@rx.uga.edu

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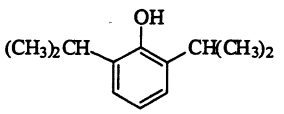
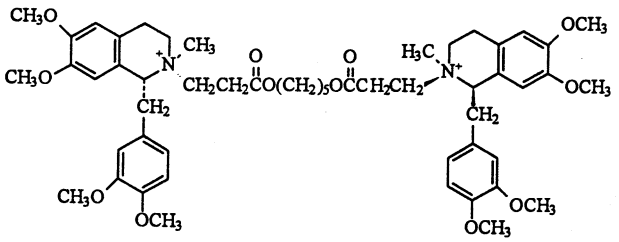


Propofol (1)



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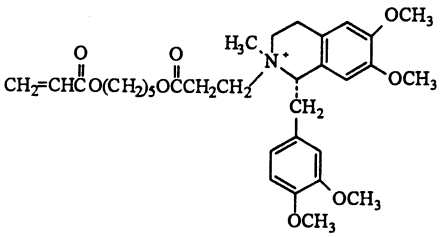
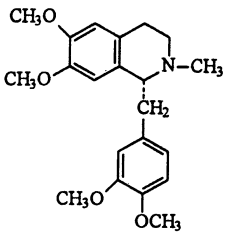
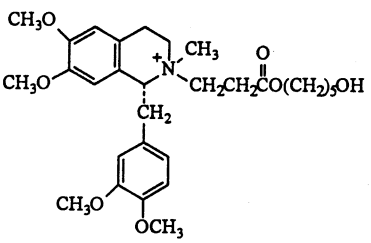


Quaternary monoacrylate (3)



Laudanosine (4)



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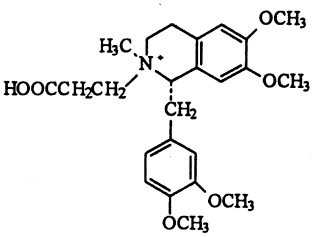

Quaternary Acid (6)

Fig. 1. Chemical structures of propofol and cisatracurium and its degradants.

in US hospitals. A stability-indicating analytical method was required for monitoring the stability and compatibility of each drug in the mixture over time.

A search of the literature showed that an analytical method was not available for the concurrent assay of cisatracurium besylate and propofol in a mixture. Cisatracurium and its metabolites or decomposition products have been assayed by HPLC with UV detection [2], fluorescence detection [3] and mass spectrometric detection [4]. These methods primarily used reverse phase chromatography with gradient elution. Propofol has been analyzed by several chromatographic methods, including GC [5–7], HPTLC [8] and HPLC [9–14]. This laboratory previously reported HPLC methods for the assay of propofol–thiopental sodium and propofol–ondansetron

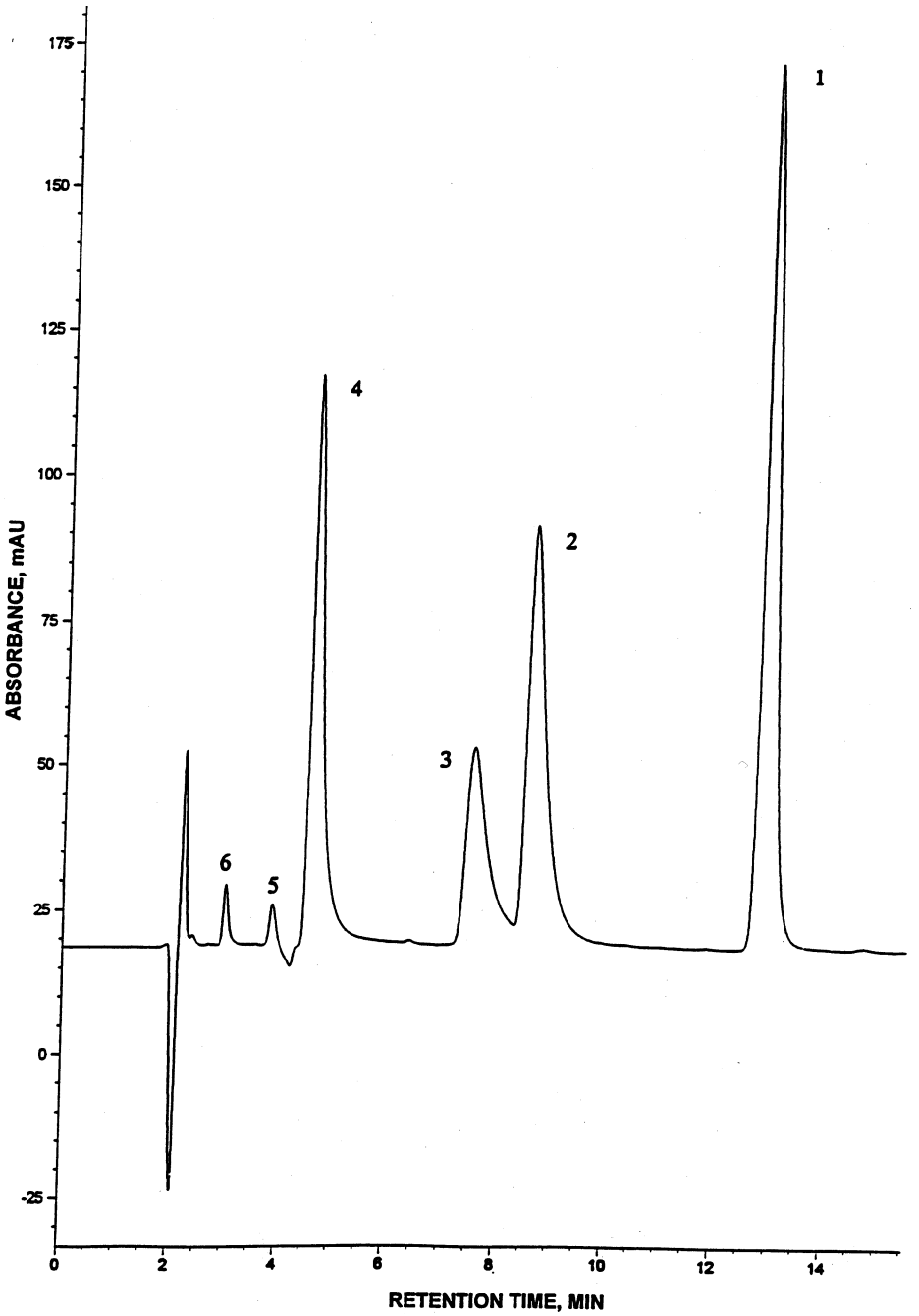


Fig. 2. A typical HPLC chromatogram of a partially degraded mixture of propofol (1) and cisatracurium besylate (2), containing quaternary monoacrylate (3), laudanosine (4), quaternary alcohol (5) and quaternary acid (6). Conditions: 250 × 4.6 mm i.d. Spherisorb ODS-2 column; acetonitrile–ammonium formate (0.3 M; pH 5.2; 50:50 v/v) with a flow rate of 1.0 ml $min^{-1}$ and detection at 280 nm.

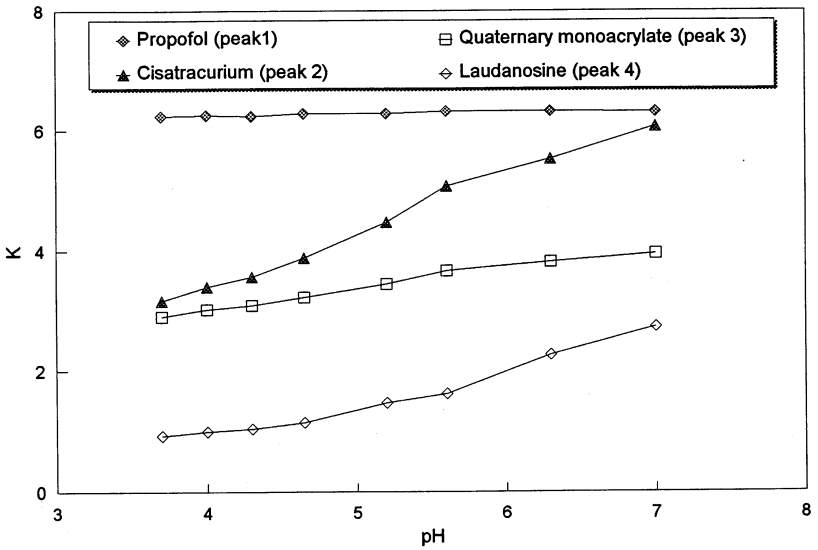


Fig. 3. Effect of pH on retention factors. Mobile phases consist of acetonitrile–ammonium formate (0.3 M; 50:50 v/v).

mixtures [9]. A comparison of HPLC to second-derivative UV spectroscopy was reported for one propofol dosage form [10]. Other HPLC procedures used to determine propofol levels in serum or plasma samples were based on reverse phase chromatography using UV or fluorescence detections [11–14].

In this paper, a simple and accurate stability-indicating HPLC assay method is presented for the quantitative determination of cisatracurium and propofol in a mixture. The identification of the degradation products of cisatracurium was based on LC-MS data.

## 2. Experimental

### 2.1. Reagents and chemicals

The cisatracurium besylate working standard was supplied by Glaxo–Wellcome (Research Triangle Park, NC). The propofol working standard was provided by Zeneca Pharmaceuticals (Wilmington, DE). HPLC grade acetonitrile was obtained from J.T. Baker (Phillipsburg, NJ) and water was purified by a cartridge system (Continental Water Systems, Roswell, GA). Ammonium formate and formic acid were obtained from Aldrich Chemical (Milwaukee, WI). The mobile phase for the HPLC system was filtered through a 0.45 μm nylon 66 filter (MSI, Westborough, MA) and degassed by sonication prior to use.

### 2.2. Instrumentation

The chromatographic system consisted of a Beckman Model 110B solvent delivery module (Beckman, San Ramon, CA), an Alcott Model 738 autosampler (Alcott Chromatography, Norcross, GA) and a Waters Lambda-Max Model 481 LC spectrophotometer (Waters Associates, Milford, MA). The detector wavelength was set at 280 nm. Data acquisition and manipulation were performed on a HP Model 3394A integrator (Hewlett–Packard, Avondale, PA).

Separation was accomplished on a $250 \times 4.6$ mm i.d. Spherisorb ODS-2 column with a particle size of 5 μm (Alltech Associates, Deerfield, IL). The column was maintained at ambient temperature ($23 \pm 1$°C). The isocratic mobile phase consisted of acetonitrile-ammonium formate (0.3 M; adjusted to pH 5.2 with formic acid) (50:50, v/v). The flow rate was set at 1.0 ml $min^{-1}$.

All LC-MS experiments were conducted on an HP Model 1100 HPLC system (Hewlett–Packard, Palo Alto, CA) coupled in series to a Micromass

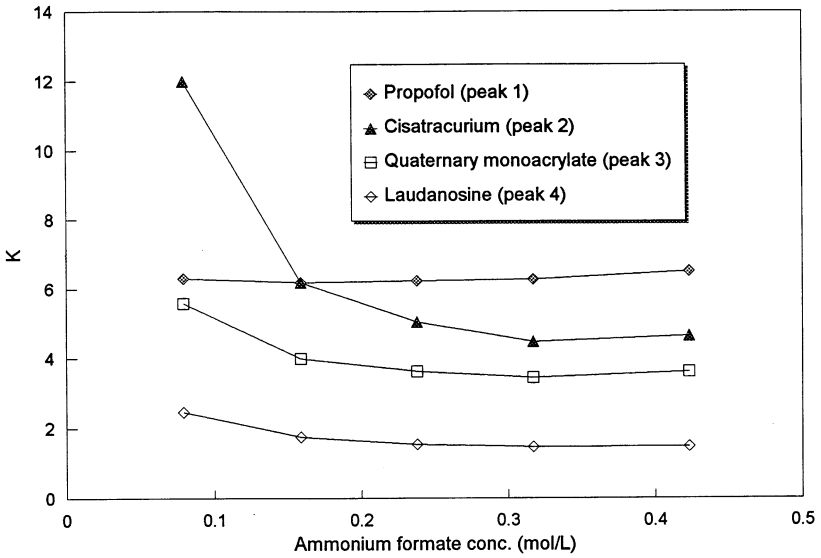


Fig. 4. Dependence of retention factors on the ionic strength of ammonium formate. Mobile phases consist of acetonitrile–ammonium formate (pH 5.2; 50:50 v/v).

Quattro II (Beverly, MA) triple quadruple mass spectrometer, which was equipped with an electrospray ionization (ESI) or atmosphere pressure chemical ionization (APcI) ion source. Full scan mass spectra were acquired over a range of $m/z$ 150–600 to identify the mass of the individual components.

### 2.3. Sample preparation

A combined stock solution containing 128 μg $ml^{-1}$ of cisatracurium besylate and 592 μg $ml^{-1}$ of propofol was prepared by dissolving weighed amounts of each reference standard in acetonitrile. The combined stock solution together with diluted aliquots of the stock solution in acetonitrile gave standard concentrations in the 8–128 μg $ml^{-1}$ and 37–592 μg $ml^{-1}$ ranges for cisatracurium besylate and propofol, respectively. Additional dilutions of the combined stock solution were prepared in acetonitrile to serve as spiked samples for the determination of accuracy and precision of the method.

All samples containing cisatracurium besylate and propofol including standard, spiked and degraded samples, were placed into autosampler vials and 20-μl aliquots were injected into the HPLC system.

## 3. Results and discussion

### 3.1. Degradation studies

The structural formulae of the two analytes and known cisatracurium degradation products are shown in Fig. 1. Mixtures of cisatracurium and propofol were degraded at ambient temperature under various conditions such as exposure to 0.01 N HCl and NaOH and 3–30% hydrogen peroxide. As shown in Fig. 2, four degradation products (peaks 3–6) of cisatracurium (peak 2) were detected under rather mild conditions (e.g. 0.01 N NaOH for 1 min). Under more extreme conditions (e.g. 0.01 N NaOH for 1 h), further degradation resulted in the complete disappearance of the cisatracurium peak and peak 3, while peaks 4–6 intensified. Little or no degradation of cisatracurium was observed with 0.01 N HCl and 3–30% hydrogen peroxide over time at ambient temperature. There were no propofol (peak 1) degradation products observed under any of the

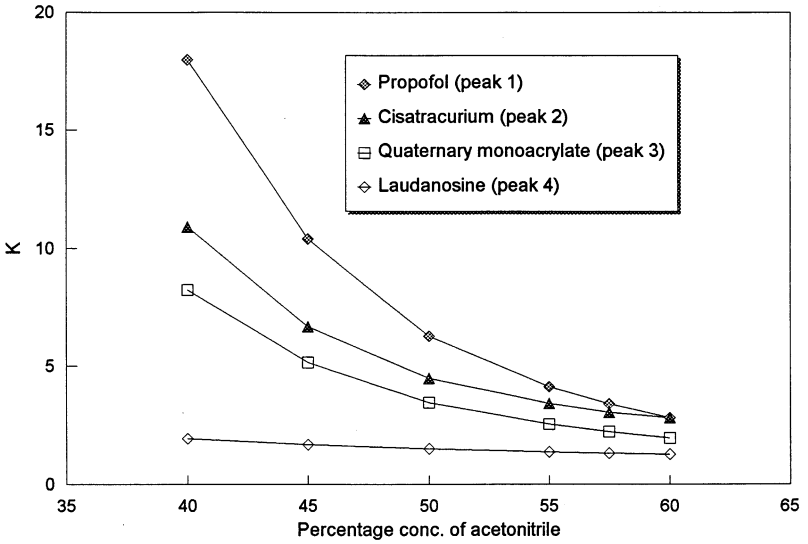


Fig. 5. Retention factors as a function of acetonitrile concentration. Mobile phases consist of acetonitrile and ammonium formate (0.3 M; pH 5.2).

conditions employed. In order to develop a stability-indicating method, mild conditions were employed to prepare a partially degraded mixture for the optimization of the HPLC separation.

### 3.2. Separation studies

Octadecylsilane and octylsilane columns were initially investigated. It was found that the retention times of the cisatracurium and propofol peaks were too far apart on the octylsilane column. In order to decrease the total assay time and maintain the desired resolution, an octadecylsilane column was used in this study. An ammonium formate buffer was used in the mobile phase so that each drug and its degradation products could be identified by LC-MS.

The effect of the pH of the mobile phase on the separation of the analytes was investigated by varying the pH of aqueous ammonium formate from 3.7 to 7.0 while maintaining the ionic strength of the ammonium formate and the composition of the mobile phase (Fig. 3). The retention factor of propofol was not affected by pH, while that of cisatracurium and its two major degradation products (peaks 3 and 4) varied. The best separations occurred over the pH range of 5.2–6.3. Since cisatracurium was more stable in acid solution, a pH of 5.2 was selected for further method development.

The effect of the ionic strength of ammonium formate was also investigated by varying its concentration in the mobile phase. As shown in Fig. 4, the propofol retention factor remained constant with different ionic strengths of ammonium formate, whereas there was a reduction in the retention factor of cisatracurium with increasing ionic strength of ammonium formate. There were also minor decreases in retention factors for peaks 3 and 4, as well as significant band broadening for peaks 2, 3 and 4 at lower ionic strengths of ammonium formate. Therefore, 0.3 M ammonium formate was selected for use in the mobile phase to obtain both better separation and resolution of the various peaks.

The chromatographic results observed with acetonitrile as organic modifier were significantly different from those using methanol. The analytes, especially cisatracurium and its two major degradation products, showed broad peaks and long tailing with methanol as the organic modifier. In contrast, much better resolution and less peak tailing were achieved with acetonitrile. As expected, peak capacities decreased as the volume

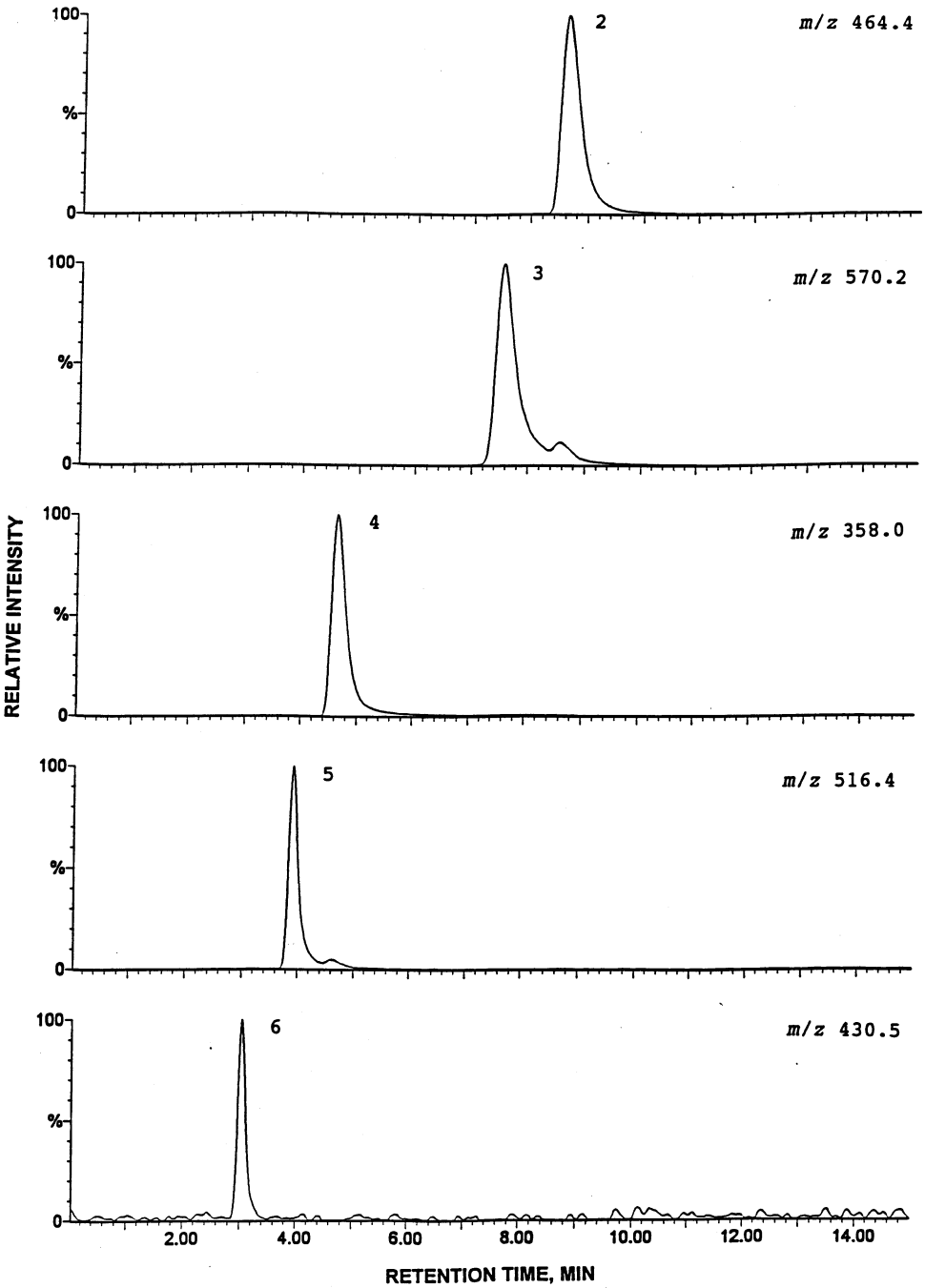


Fig. 6. Selected ion chromatograms of cisatracurium (2), quaternary monoacrylate (3), laudanosine (4), quaternary alcohol (5) and quaternary acid (6), using LC-MS with positive ESI.

Table 1
Analytical figures of merit for cisatracurium and propofol on an octadecylsilane column

| Analyte | $(r^2)$[a] | Retention factor $(K')$ | Theoretical plates $(N)$[b] | Tailing factor[c] | Resolution (Rs) |
|---|---|---|---|---|---|
| Cisatracurium | 0.9998 | 4.48 | 2300 | 1.20 | 4.30 |
| Propofol | 0.9999 | 6.11 | 9840 | 1.03 | |

[a] Range examined from 8–128 μg ml$^{-1}$ cisatracurium besylate ($n = 6$) and 37–592 μg ml$^{-1}$ propofol ($n = 6$). The mobile phase consisted of acetonitrile–ammonium formate (pH 5.2; 0.3 M; 50:50 v/v) at 1.0 ml min$^{-1}$ with detection at 280 nm.
[b] Calculated as $N = 16\ (t/w)^2$.
[c] Calculated at 5% peak height.

ratio of the organic modifier increased in the mobile phase (Fig. 5). A 50% volume of acetonitrile in the mobile phase was preferred for a shorter run time and adequate resolution. Thus, a mobile phase consisting of acetonitrile–ammonium formate (pH 5.2; 0.3 M; 50:50 v/v) was selected for the assay. A typical HPLC chromatogram of a partially degraded mixture of cisatracurium besylate and propofol is shown in Fig. 2.

### 3.3. Identification of analytes and degradation products

In the process of optimizing the separation conditions for the mixture, peaks 3 and 4 showed similar chromatographic characteristics to cisatracurium. It was reported in the literature that cisatracurium undergoes pH and temperature dependent chemical degradation, commonly referred to as Hofmann elimination, to form laudanosine and a quaternary monoacrylate [3]. It also undergoes ester hydrolysis to form a quaternary alcohol and a quaternary acid (see structures 3–6 in Fig. 1). It appeared from our studies that peaks 3 and 4 were the principal degradation products of cisatracurium.

Using LC-MS, the peaks of the degraded analyte mixture were identified by their mass-to-charge ratios. Samples were analyzed by ESI in the positive-ion mode over the mass range from $m/z$ 150 to 600. Cisatracurium (peak 2) was observed as a doubly charged molecular species at $m/z$ 464 by virtue of its two quaternary nitrogens. Peak 3 was observed at $m/z$ 570 as a singly charged quaternary monoacrylate and peak 4 was detected as a protonated laudanosine molecule at $m/z$ 358. Two small peaks close to the solvent front were identified as the cisatracurium ester hydrolysis products, peak 5, the quaternary alcohol and peak 6, the quaternary acid. Both were determined as their singly charged molecular species at $m/z$ 516 and 430, respectively (Fig. 6).

Propofol ($m/z$ 178) was not observed with ESI in the positive-ion mode because it was difficult to protonate. Peak 1 was confirmed as propofol both by its chromatographic retention time and by mass spectrometric detection using APcI in the negative-ion mode [15].

### 3.4. Analytical figures of merit

Determination of cisatracurium besylate and propofol in spiked mixtures was performed using external calibration. Calibration curves were generated by least-square regression of the analyte peak areas against concentration. The regression analysis showed linearity for cisatracurium and propofol over the 8–128 and 37–592 μg ml$^{-1}$ ranges respectively, at 280 nm. Table 1 gives analytical figures of merit for each analyte.

Percent error (accuracy) and precision of the method were evaluated using the spiked samples containing each analyte. The data shown in Table 2 indicated that the procedure gave acceptable accuracy and precision for both analytes.

## 4. Conclusions

A simple and accurate stability-indicating HPLC method has been developed for the determination of cisatracurium besylate and propofol

Table 2
Accuracy and precision using spiked drug samples

| Analyte | Concentration added (μg ml$^{-1}$) | Concentration found (μg ml$^{-1}$) | Percent error | RSD (%) | *n* |
|---|---|---|---|---|---|
| Intra-day | | | | | |
| Cisatracurium | 21.97 | 22.22 ± 0.64 | 1.1 | 2.9 | 6 |
| Besylate | 87.87 | 87.45 ± 1.22 | 0.5 | 1.4 | 6 |
| Propofol | 69.41 | 69.03 ± 2.00 | 0.5 | 2.9 | 6 |
| | 277.6 | 281.6 ± 2.50 | 1.4 | 0.9 | 6 |
| Inter-day | | | | | |
| Cisatracurium | 21.97 | 22.20 ± 0.22 | 1.0 | 1.0 | 5 |
| Besylate | 87.8 | 86.76 ± 0.35 | 1.3 | 0.4 | 5 |
| Propofol | 69.41 | 69.67 ± 0.77 | 0.4 | 1.1 | 5 |
| | 277.6 | 279.0 ± 1.94 | 0.5 | 0.7 | 5 |

The number of days for the inter-day study and the number of samples analyzed on one day for the intra-day study are represented by *n*.

in mixtures. Degradation products resulting from pH and time stress were separated from cisatracurium and propofol and identified by mass spectrometry as Hofmann elimination and ester hydrolysis products of cisatracurium. There were no propofol degradation products observed. The assay method should be suitable for monitoring the stability and concentration of cisatracurium besylate and propofol in mixtures over time.

## Acknowledgements

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