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Short communication

Simultaneous detection of cisatracurium, its degradation products and propofol using positive ion detection followed by negative ion detection in a single LC/MS run

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1. Introduction

Cisatracurium (Nimbex®), a non-depolarizing skeletal muscle relaxant for intravenous administration, is one of ten optical and geometric isomers in atracurium and represents ~14% of this mixture. Cisatracurium is ~1.5 times more potent than atracurium and unlike atracurium, does not cause the release of histamine at a dose 60 times the ED_{95} neuromuscular blocking dose (ED_{95} is the dose required to produce 95% suppression of the adductor pollicis muscle twitch response to ulnar nerve stimulation). In addition, no cardiovascular side effects, flushing, or bronchospasms are observed in patients receiving cisatracurium. Welsh et al. report Hoffmann elimination at physiological pH and temperature inactivates the mixture of isomers in atracurium resulting in a series of degradation products [1]. Cisatracurium besylate also slowly loses potency at a rate of ~5% per year under refrigeration (5°C). The manufacturer recommends that cisatracurium besylate be refrigerated at 2–8°C in order to preserve potency. The rate of loss of potency increases to ~5% per month at a temperature of 25°C [2].

Propofol (2,6-diisopropylphenol), an intravenous anaesthetic agent, has been commonly used with cisatracurium in hospitals. Propofol is frequently used for both induction and maintenance of anaesthesia during surgical procedures in intensive care units (ICU). Intravenous injection of a therapeutic dose of propofol produces hypnosis rapidly with minimal excitation, usually within 40 sec from the start of an injection (the time for one arm-brain circulation) [3,4].

There have been several reports on the analysis of cisatracurium using HPLC with UV detection [5–8,16], fluorimetric detection [9–13] and mass spectrometric detection [14]. The decomposition products of cisatracurium have also been analyzed

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by HPLC [1,6,7,15]. Propofol has been analyzed by HPLC with UV detection [17–21], electrochemical detection [22–24], fluorescence [25–27], GC/MS [28,29] and GC-FID [30].

We have recently developed an assay to separate and identify a mixture of cisatracurium, its degradation products and propofol simultaneously [31]. However, while we were analyzing this mixture, we did not observe a response for propofol in positive electrospray ionization (ESI) mode due to the chemical properties of propofol. Even during flow injection of a high concentration (20 ppm) of the propofol standard, the protonated pseudomolecular ion, $(M+H)^+$, was not observed. However, the deprotonated propofol signal, $(M-H)^-$, was abundant under negative ionization mode. Since propofol has the longest retention time on a non-polar C_{18} column compared to cisatracurium and its degradation products, it was possible to switch the detection polarity in the middle of the LC run allowing cisatracurium, its degradation products and propofol to be detected simultaneously in a single LC/MS run. The selection of detection modes (positive and negative) is strongly dependent on the chemical properties of the analytes [32,33]. Some compounds are easily protonated such as proteins and peptides due to the amine group while some compounds are readily deprotonated such as nucleotides due to the phosphate group. Van Breemen et al. [34] have reported the separation of active retinoids in vitamin A using HPLC followed by mass spectrometric detection. They report that retinol, retinal and retinyl acetate were detected in positive ion mode but not in negative ion mode, while retinoic acid produced a strong deprotonated signal in the negative ion mode while no ions were observed in the positive ion mode. Barnes et al. recently reported the determination of ten pesticides using ionization polarity switching [35] and APcI LC/MS. In their experiment, the first eight components were detected in positive ion mode, while the final two components were detected in negative ion mode.

In pharmaceutical analysis, one is usually interested in analyzing for a single drug and per haps a number of structurally related compounds such as metabolites. However, with the increase in the use of combination therapies, the analysis of multiple drugs in a single assay is becoming more frequent and often times provides quite a challenge. Since most pharmaceuticals are basic by nature the majority tend to run efficiently in positive ion mode, but in those cases where the monitoring of both acidic and basic drugs is necessary the use of both positive ion and negative ion detection in a single run becomes an attractive alternative.

2. Experimental

2.1. Chemicals

The cisatracurium besylate ([1R-[1α,2α(1′R*, 2′R*)]] - 2,2′ - [1,5 - pentanediylbis[oxy(3 - oxo - 3,1 - propanediyl)]]bis[1 - [(3,4 - dimethoxyphenyl) - methyl] - 1,2,3,4 - tetrahydro - 6,7 - dimethoxy - 2 - methylisoquinolinium] dibenzenesulfonate) standard was provided by Glaxo-Wellcome (Research Triangle Park, NC) and the propofol (2,6-diisopropylphenol) working standard was supplied by Zeneca Pharmaceuticals (Wilmington, DE). The structural formulas of both compounds are shown in Fig. 1. Methanol (HPLC grade, J.T. Baker, Philipsburg, NJ), acetonitrile (HPLC grade, J.T. Baker, Philipsburg, NJ), formic acid (88%, J.T. Baker, Philipsburg, NJ) and ammonium formate (97 + %, Aldrich, Milwaukee, WI), were used without further purification. Deionized water was generated from a Continental Deionized water system (Natick, MA).

2.2. Instrumentation

High performance liquid chromatographic (HPLC) separations of samples were achieved on a Hewlett-Packard (Palo Alto, CA) Model 1100 system controlled by an HP Chem Station (Rev. A04.01) data system. The injection volume was 20 μl and the mobile phase consisted of 50:50 (v/v) 0.3 M ammonia formate (pH adjusted to 5.6 by formic acid)-acetonitrile. The

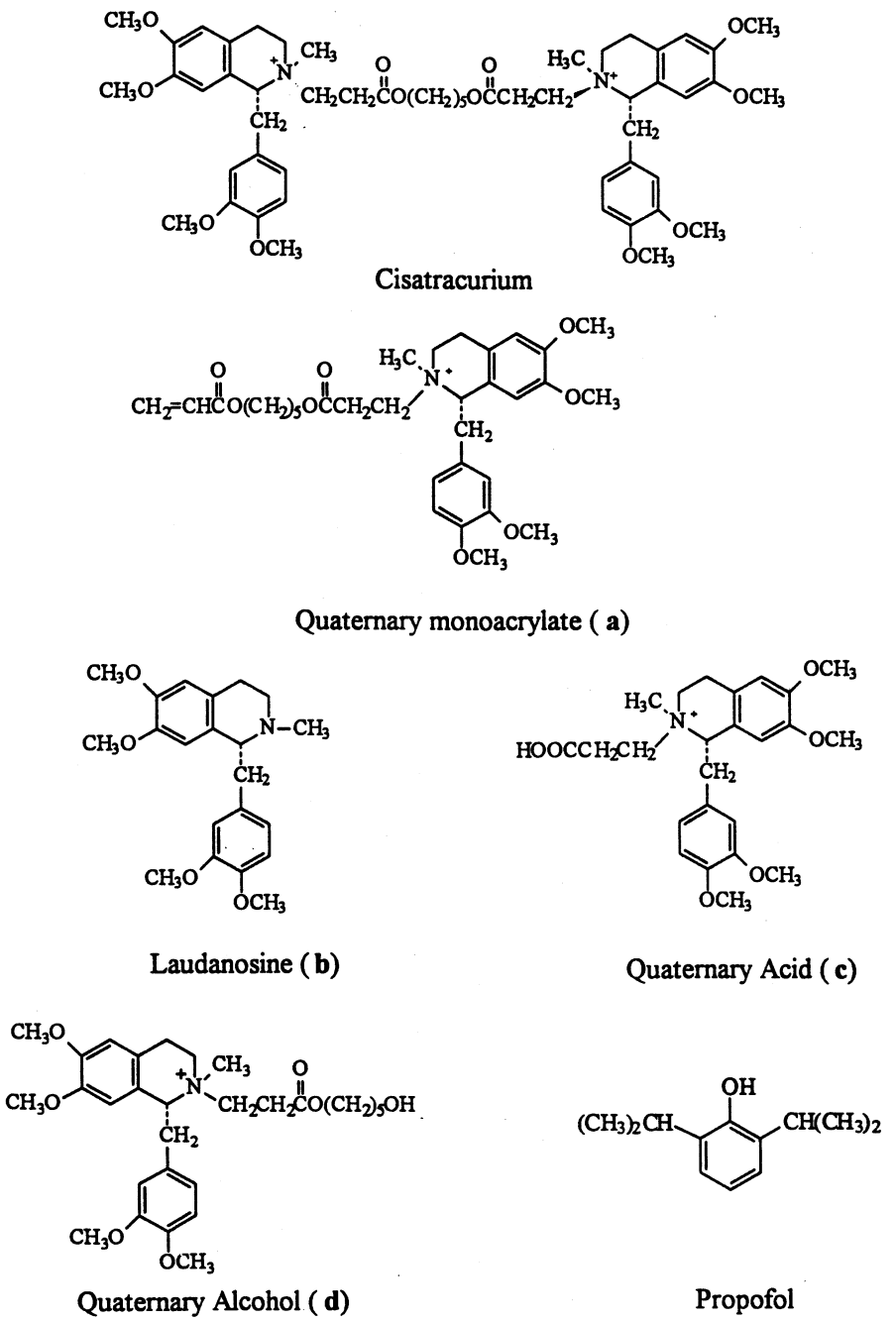

Fig. 1. Chemical structures of cisatracurium, its degradation products and propofol.

flow rate of the mobile phase was 1.0 ml min^{-1} and the 250×4.6 mm i.d. Spherisorb ODS-2 column with 5 μm particle size (Alltech Associates, Deerfield, IL) was heated to 40°C. The analytes were monitored at a wavelength of 280 nm.

Mass spectrometric experiments were performed with a Micromass Quattro II (Beverly, MA) triple quadruple mass spectrometer equipped with an ESI or atmosphere pressure chemical ionization (APcI) ion source. For ESI experiments, the source temperature was set to 150°C, ion source capillary and cone voltages were adjusted to maximize the primary ion beam intensity, but were generally in the range of 2500–3500 V and 25–50 V, respectively. For APcI experiments, the source temperature was set to 150°C and the APcI probe temperature was set to 550°C. Corona and cone voltages were set to 2500 V and 35 V, respectively. All LC/MS data was recorded in continuum mode. The mass resolution was set to unit mass resolution.

Flow injection studies were performed by delivering sample solutions via a syringe infusion pump (Model 200, KD Scientific, Boston, MA) at a flow rate of 10 μl min^{-1} into the ion source through a 6-valve injector (Rheodyne, Cotati, CA) with a 100 μl sample loop.

3. Results and discussion

3.1. LC/MS determination of cisatracurium and propofol

To optimize the signal intensities of LC/MS, both ESI and APcI techniques were explored. We observed that ESI and APcI were similar in terms of absolute signal intensity for cisatracurium and its degradation products. However, for propofol, APcI produces a much stronger signal in the negative ionization mode. The is due to the fact that propofol is a less polar compound and APcI tends to give better sensitivity for these types of compounds [36]. Therefore, in this study, APcI ionization mode was used unless mentioned otherwise.

The separation of cisatracurium and its degradants with propofol was achieved in this lab and will be published elsewhere [31]. The degradation products of cisatracurium were identified by LC/MS with MS detection set in the positive mode which allowed the quaternary ions or protonated molecules to be detected. To optimize the ionization conditions of both cisatracurium and propofol, flow injection of both cisatracurium and propofol individually was needed. With the mobile phase of methanol or 50:50 (v/v) 0.3 M ammonia formate (pH = 5.6)/acetonitrile, cisatracurium was only observed in positive ion mode and propofol was only observed in negative ion mode (deprotonated form). Both positive and negative ion full-scan mode experiments over the mass range from m/z 150 to 600 were used to determine the m/z of the individual components related to cisatracurium, its degradation products and propofol, then SIR (single ion recording) mode was used to monitor the positive ions m/z 570.2, 516.4, 464.4, 430.5 and 358.0 and the negative ion 177.0. The chemical structures of the degradation products of cisatracurium are shown in Fig. 1. Propofol was not detected in the positive ion mass chromatogram although it was observed in the UV trace. The reason is that under the optimized separation conditions, propofol is difficult to protonate due to stereo effects. The addition of a proton to the oxygen adjacent to the benzene ring is not favorable. Also, we observed that the deprotonated propofol [$(M-H)^-$] signal was much stronger when using methanol as the mobile phase as opposed to using the LC mobile phase of 50:50 (v/v) 0.3 M ammonia formate (pH = 5.6)/acetonitrile. The reason is because the buffered mobile phase is acidic (pH = 5.6) therefore, deprotonation of propofol is suppressed. The LC/MS trace using this isocratic mobile phase showed a weak deprotonated propofol peak when the ionization mode was switched from positive to negative mode at 11 min. To increase the ion intensity of propofol, we applied a gradient system (Table 1) to this separation. By in-

Table 1
The gradient table used for the separation of cisatracurium and propofol

Time (min)	50:50 (v/v) 0.3 M ammonium formate (pH = 5.6)/acetonitrile (%)	and methanol (%)
0	100	0
9	100	0
11	0	100
15	0	100

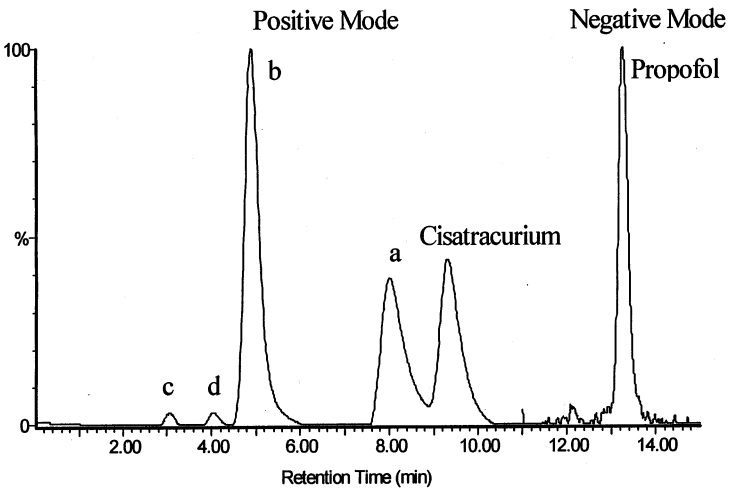

Fig. 2. LC/MS total ion chromatogram using gradient mobile phase system. Refer to Fig. 1 for the peak labels. The detection mode was switched from positive to negative at 11 min.

creasing the percentage of methanol to 100%, the deprotonated propofol signal was enhanced by a factor of 4 (Fig. 2).

3.2. Optimization of experimental parameters for an ion polarity switching assay

The variables effecting an ion polarity switching assay are the same as those that would effect any LC/MS analysis. The most important being the composition of the mobile phase at the time the analyte of interest is passing into the mass spectrometer. These include, but are not limited to, the solution pH, composition (% organic/aqueous), buffer identity and buffer concentration. Source tuning parameters such as the cone voltage may also have a substantial effect on signal intensity, although the cone voltage can be changed at any time during an individual LC/MS run and therefore is easily optimized for each individual component of a mixture. For electrospray LC/MS experiments, the needle voltage and the presence of co-eluting peaks can also have a strong influence on the response of an individual component since electrospray is sensitive to the ionizability of a compound in the solution state. Atmospheric pressure chemical ionization converts gas-phase neutral species into ions using a corona discharge, and therefore, is not as susceptible to the dramatic changes in MS response with changes in mobile-phase composition that are often observed when using ESI.

In their assay for determination of pesticides, Barnes et al. [35] found no need to alter the mobile phase composition during the analysis. Although the response for the positively charged species was significantly stronger than the response of the negatively charged species they were still able to analyze samples over the concentration range of interest. The authors did report an improved response when using APcI instead of ESI which means that a large portion of the molecules they were analyzing were not charged in their mobile-phase. We found that for cisatracurium, which is a quaternary amine and thus exists precharged in solution, both ESI and APcI worked equally well. However, propofol was difficult to deprotonate in solution and therefore required APcI to aid in detection. In our assay, the positively charged cisatracurium has a limit-of-detection of ~ 100 pg ml^{-1} while the negatively charged propofol has a limit-of-detection of 10 ng ml^{-1}. It is difficult to make really dramatic changes in certain parameters of the HPLC mobile phase, especially pH, since many columns have restrictions. Therefore, the increased ruggedness of APcI versus ESI, especially in any assay that would use ionization polarity switching, makes it the ionization technique of choice.

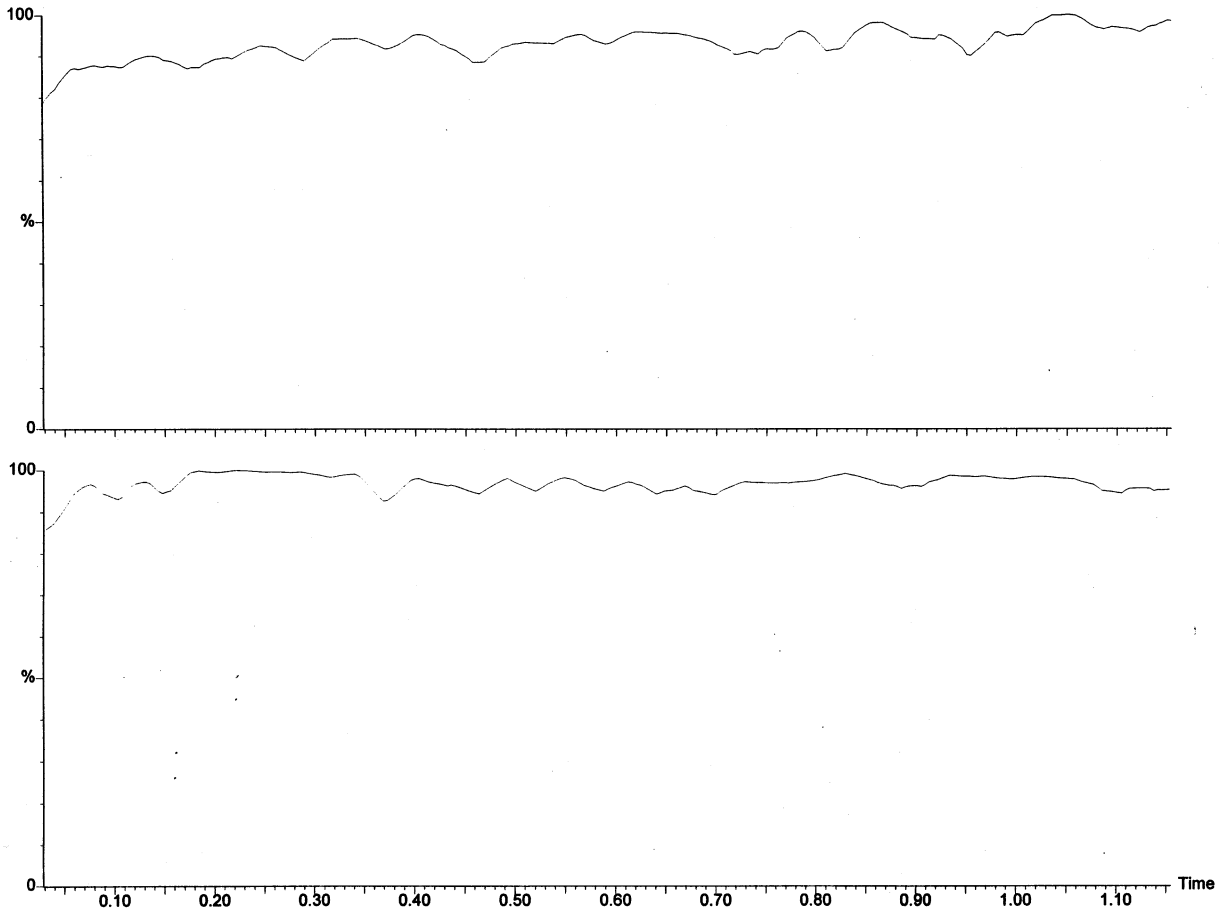

Fig. 3. Flow injection of cisatracurium and propofol mixture followed by selected ion monitoring for $(M+2H)^{2+}$ from cisatracurium (top) at m/z 464.2 and $(M-H)^{-}$ from propofol (bottom) at m/z 177 by switching the detector polarity every 0.05 s.

We have also studied several instrumental parameters that could effect ion polarity experiments. Since our assay involves detection of cisatracurium and its degradation products prior to switching to negative ion mode for detection one obvious question is how easy is it to switch between ion polarities. In our software you can switch between ion polarities as rapidly as 0.05 s and the baseline is remarkably stable to these changes. Fig. 3 shows the measurement of the doubly-charged peak from cisatracurium and the deprotonated form of propofol from the infusion of this mixture. This experiment represents a worst case scenario where there are co-eluting peaks and the need to detect both products in the same experiment. The instrument is set to measure cisatracurium for 0.05 s and then detect propofol for 0.05 s before repeating the cycle. This system allows for ten measurements of each compound to be made every second. It is worthy to note that the ion currents are quite stable and that there is no evidence for any instability in the ion source. These constant ion signals would allow for facile quantitation of these two species.

4. Conclusion

We have developed an LC/MS method to detect cisatracurium, its degradation products and

propofol in an single LC/MS run by switching ionization polarity in the middle of the LC run, therefore, analytes with different chemical properties can be detected simultaneously. This method may be applied to other applications for which a single ionization mode does not meet the requirements for detection. The ionization and detection polarity can also be switched back and forth during a single LC/MS analysis.

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