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A new method for the quantitation of propofol in human plasma: efficient solid-phase extraction and liquid chromatography/APCI-triple quadrupole mass spectrometry detection

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Abstract

Propofol (2,6-diisopropyl phenol) is widely used for the induction and maintenance of anesthesia. Analyses of its pharmacokinetics require simple and sensitive methods for quantitation of propofol in human plasma. Previously reported HPLC and GC methods are limited by cumbersome extraction steps. We describe a novel method that combines sample preparation by solid-phase extraction (SPE) with hydrophilic–lipophilic balance cartridges and analysis with a sensitive LC-APCI-triple quadrupole mass spectrometry (MS/MS) method for better quantitation.

The absolute recovery of the analyte was greater than 96%. The limit of quantification for propofol in plasma at a signal-to-noise ratio of 10 was 5 ng/ml. The precision of the assay yielded coefficients of variation ranging from 2.9 to 5.3% and an accuracies of 99–105%.

Our method advances the quantitative analysis of propofol in human plasma by combining simple, rapid and efficient SPE with specific and sensitive quantitation by HPLC with APCI-MS/MS detection.

Keywords: Propofol; Human plasma; Solid-phase extraction; Liquid chromatography; Tandem mass spectrometry

1. Introduction

Propofol (2,6-diisopropyl phenol) is an intravenous anesthetic with a phenolic structure. It is used for both induction [1] and maintenance [2] of anesthesia. It is also useful for sedation [3] in patients under regional anesthesia and in critically ill patients confined to intensive care units. After receiving a single intravenous dose of propofol, the patient loses consciousness within 30–50 s and remains unconscious for about 4–6 min [2,3]. Despite rapid redistribution, propofol is present in the blood and tissues for a few hours even after a single dose, its concentration decreasing with time [4]. To carry out pharmacokinetic/pharmacodynamic analyses involving propofol, the development of a precise analytical method for the determination of plasma propofol concentrations that is suitable for routine analysis would represent an excellent addition to the analytical armamentarium of anesthetic drugs.

Various methods have been reported for the quantitation of propofol in plasma or blood: high pressure liquid chromatography (HPLC) with ultraviolet (UV) [5,6], fluorescence [7] or electrochemical detection [8]; gas chromatography (GC) with atomic emission [9] or mass spectrometric (MS) detection [10]; and capillary GC or head space GC [11] with solid-phase microextraction. Sample preparation prior

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to analysis by HPLC or GC requires either liquid–liquid or solid-phase extraction (SPE). Liquid–liquid extraction with various solvents, although frequently used [12–16], is tedious and time–consuming. Some methods show good recovery of propofol, but their sensitivity is constrained by interfering constituents of plasma. Alternatively, during concentrating steps, propofol may be lost due to its volatile nature [7]. Published methods of SPE by reversed phase C18 cartridge are limited by incomplete removal of plasma constituents, suboptimal recovery and variable reproducibility [6,8]. In this paper we are reporting a novel LC/MS/MS method for the detection and quantitation of propofol in human plasma. The specificity of detection by triple quadrupole MS compared to traditional modes of detection reduced the need for complete resolution of the analyte during extraction and separation steps allowing sample preparation by rapid and efficient SPE with hydrophilic–lipophilic balance (HLB) cartridges.

2. Experimental

2.1. Drugs and chemicals

Propofol and the internal standard thymol were purchased from Sigma (St. Louis, MO). HPLC-grade methanol and aqueous ammonium hydroxide were obtained from Fischer Scientific (Hampton, NH). Water was filtered with a nanopure system (Barnstead, Dubuque, Iowa). The mobile phase was passed through a 0.22 μm nylon filter (Millipore, Bedford, MA) and degassed by sonication. Oasis hydrophilic–lipophilic balance (HLB) cartridges (1 ml; 30 mg) were purchased from Waters (Milford, CT). Drug free normal human plasma was purchased from the local blood bank (Civitan Regional Blood Center, Gainesville, FL). Samples were stored at 4 °C until analyzed.

2.2. Standard solution and calibration standards

Stock solutions (10 mg/ml) of propofol and thymol were prepared in methanol and stored at 4 °C. Vials were filled with nitrogen after opening to prevent drug oxidation. Standard solutions of propofol (2, 20 and 200 μg/ml) were made by further dilution of the stock solution with methanol. Quality control samples (10, 100 and 1000 ng/ml) were prepared by adding 50 μl of appropriate propofol standard solution to 10 ml of blank plasma. These were vortexed and kept frozen in 1 ml aliquots at −20 °C until analysis.

For calibration curves, blank plasma samples (1 ml) were spiked with 25 μl of the appropriately diluted standard solutions to final propofol concentrations of 5, 10, 50,100, 500, 1000 and 2000 ng/ml. Control samples containing no added propofol were also prepared. All samples were subjected to the sample preparation procedure as described below. Accuracy and precision of the assay were determined by generating intra- and inter-day variability data from a series of samples in the range of 10–1000 ng/ml, either extracted and injected three times on a single day or on three different days, respectively. The recovery of propofol was determined by comparing the slopes of calibration curves from extracted samples with those from corresponding unextracted samples prepared in mobile phase. This approach was chosen, because a separate series of preliminary experiments demonstrated that recovery of internal standard consistently exceeded 95% (data not shown).

2.3. Extraction procedure

SPE cartridges were activated with 1 ml of methanol and washed with 1 ml of water. Plasma samples (1 ml) were diluted with 1 ml of phosphate-buffered saline after adding 20 μl of thymol (50 μg/ml) as an internal standard, acidified with 20 μl of phosphoric acid, vortexed, and loaded onto activated cartridges. The cartridges were washed three times (1 ml of water followed by 1 ml of 1% $KHCO_3$ in acetonitrile:water (1:9), followed by 1 ml of acetonitrile:water (2:3)) and dried in a low vacuum for 30 s. Solutes were eluted with 1 ml of methanol without vacuum. Vacuum was used briefly to remove remaining solvent in the cartridge. Solutes were vortexed and transferred to autosampler vials for analysis.

2.4. Equipment

LC/MS/MS analyses were performed using a Perkin-Elmer PE 200 LC system consisting of two Series 200 pumps, one Series 200 autosampler, a two port diverter valve (Valco Instruments, Houston, TX) and an API 4000 triple quadrupole mass spectrometer equipped with an APCI source (Applied Biosystems, Foster City, CA), run by analyst software (version 1.3.1).

2.5. HPLC conditions

Samples (25 μl injection volume) were separated on a C18 column (Xterra TM RP18, ODS 5 μm 250 mm × 4.6 mm i.d., Waters) with a guard cartridge (4 mm × 3 mm i.d., Phenomenex, Torrance, CA) prior to the C18 column. The isocratic mobile phase consisted of methanol and 0.05% aqueous ammonium hydroxide solution (98:2) at a flow rate of 1 ml/min. To avoid soiling of the mass spectrometer with matrix molecules, the effluent was initially directed to the waste for 2.9 min with the help of a diverter valve. Then the valve was automatically switched to direct the effluent to the mass spectrometer.

2.6. Mass spectrometric conditions

In the MS/MS experiments, the deprotonated precursor molecular ions were selected and fragmented by nitrogen gas collision in the Q2 region with a collision energy of −45 eV. The resulting mass spectra were acquired in full scan mode from *m/z* 100–200. Several product ions were observed for both propofol and thymol. The most abundant product ion at

161 for propofol and the ion at 133 for thymol were selected for multiple reaction monitoring (MRM) quantitation. Operating conditions for the APCI source used in the negative ion mode were set to a vaporizer temperature of 450 °C, corona discharge intensity of 5 μA, with a gas flow of 48 units (units refers to an arbitrary value set by the analyst software).

2.7. Statistical analysis

Calibration curves were constructed by plotting the ion abundance peak area ratios (propofol/IS) as a function of plasma propofol concentration. These data were then fitted with an unweighted least squares regression analysis to the equation: $y = ax + b$, where a defines the slope and b the intercept of the ordinate. The propofol concentrations of unknown samples were calculated using the results of the regression analyses. The data are expressed as mean value ± S.D.

3. Results and discussion

3.1. Recovery of propofol

Sample preparation by SPE with HLB cartridges effectively purified the plasma sample while providing good recovery of propofol and thymol. Initially, during method optimization various solvents were tried for the extraction of propofol from the HLB cartridge (Fig. 1). Recovery for methanol as the solvent was significantly better than for either acetonitrile, ethyl acetate, cyclohexane or chloroform. Since propofol is very lipophilic and extensively bound to plasma proteins, phosphoric acid was added to overcome binding and improve recovery beyond 80%. Subsequent washing with a 1% $KHCO_3$ solution removed any traces of phosphoric acid, which otherwise might suppress the ionization of propofol in the negative ion mode. The recovery of propofol was determined over a wide concentration range by comparing the measured concentrations of plasma containing propofol from 5 to 1000 ng/ml with those of equivalent standards prepared in methanol and injected directly into the column. As shown in Table 1, the mean absolute recovery of propofol exceeded 95% at all propofol concentrations.

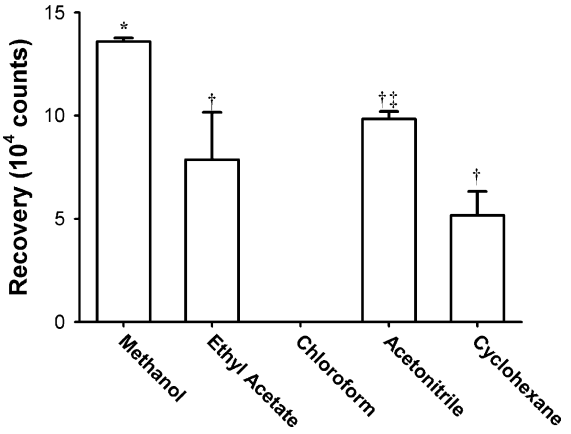

Fig. 1. Solvent effects on recovery of propofol after solid-phase extraction with HLB cartridges. Samples contained propofol 100 ng/ml. Data is mean ± S.D. of triplicate determinations. *$p < 0.05$ vs. all other solvents; †$p < 0.05$ vs. chloroform; ‡$p < 0.05$ vs. cyclohexane (ANOVA with Tukey post-hoc testing).

SPE with HLB cartridges compares favorably with previous methods of sample preparation. Liquid–liquid extraction of propofol, which is described in most previous reports [12–16], extracted many unrelated compounds along with propofol thus increasing the background for the detector. Reports describing SPE of propofol from plasma [6,8] utilized SPE by a reversed phase C18 cartridge, which has two disadvantages: (1) the overall recovery may be as low as 72% [8], and (2) reproducibility is variable, particularly if the cartridge runs dry during extraction. The Oasis HLB cartridge, which has been used widely for the extraction of drugs from biological matrices, allowed us to overcome these problems. Comparison of chromatograms from unextracted standard solution with extracted plasma samples clearly shows no interfering peaks at the propofol retention time (Fig. 2).

In order to maximize sensitivity, we attempted to obtain a more concentrated sample. Due to the volatile nature of propofol, which forms the basis for head space GC [11] but causes sample loss during concentration after the extraction step [8], we did not attempt to concentrate the sample by evaporation. Plummer [7] used a quaternary ammonium salt to make the organic phase basic and thereby prevent loss of propofol and internal standard during the evaporation step. This approach, however, was not suitable for sample preparation prior to LC/MS analysis, because quarternary alkyl amines are difficult to remove from the MS [17]. Therefore, we attempted to obtain a more concentrated sample by minimizing the amount of methanol for sample elution during the solid-phase extraction. However, less than 1 ml of methanol was insufficient to elute the compound completely from the cartridge (data not shown).

3.2. Mass spectrometry

Specific identification of propofol and thymol was done using atmospheric pressure chemical ionization (APCI) in the negative ion mode as shown in Fig. 3. Deprotonation of propofol and thymol yielded precursor ions of *m/z* 177 and

Table 1
Absolute recovery of propofol

Spiked concentration (ng/ml)	Measured concentration (ng/ml)	CV (%)	Recovery (%)
5	5.1 ± 0.3	4.9	101.9
10	10.4 ± 0.7	6.7	104.3
50	51.0 ± 1.3	2.6	102.1
100	101.5 ± 5.4	5.4	101.5
500	486.8 ± 21.0	4.3	97.4
1000	987.9 ± 35.3	3.6	98.8

Data are mean ± S.D. of triplicate determinations. CV: coefficient of variation.

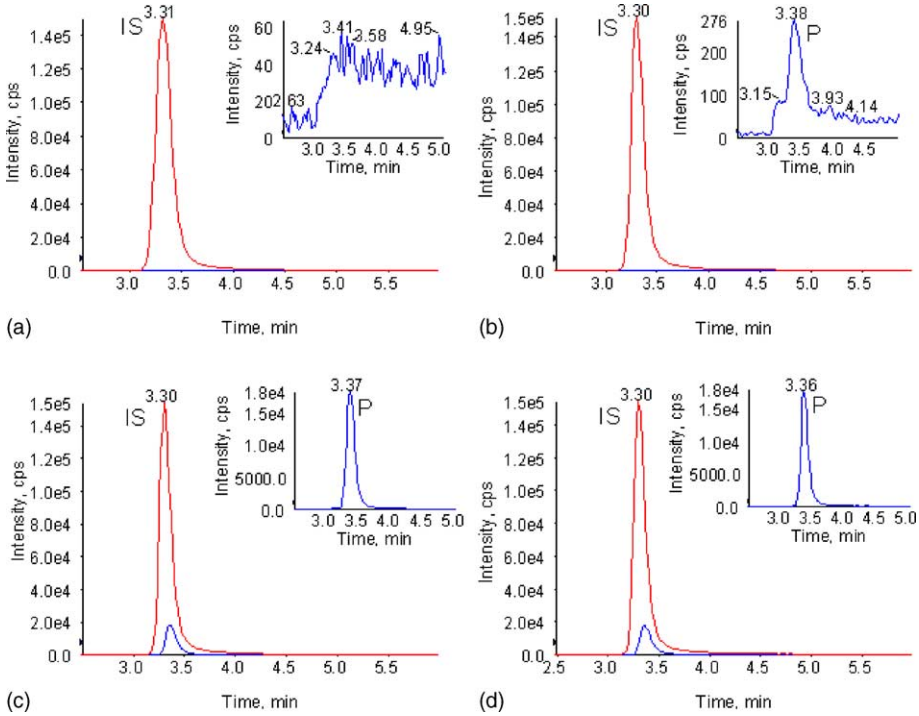

Fig. 2. Representative total ion chromatograms of propofol showing (a) blank plasma with internal standard (b) propofol in plasma at 1 ng/ml (c) propofol in human plasma at 100 ng/ml (d) propofol in methanol at 100 ng/ml. The insets show the extracted ion chromatogram from 2.5 to 5 min, thus isolating the propofol region of the chromatogram on an expanded scale (IS = internal standard, thymol; P = propofol).

m/z 149, respectively. The most intense product ions were *m/z* 161 for propofol and *m/z* 133 for thymol, suggesting the loss of a methane molecule from their corresponding precursor ions. Precursor ion scans of ions *m/z* 161 and *m/z* 133 confirmed their origin from deprotonated propofol (*m/z* 177) and thymol (*m/z* 149), respectively.

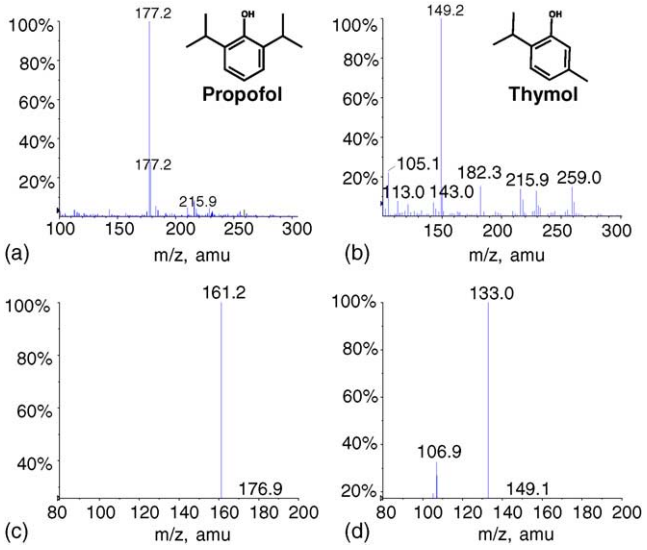

Fig. 3. Identification of propofol and the internal standard thymol by APCI-triple quadrupole mass spectrometry. (a) Propofol parent ion scan; (b) thymol parent ion scan; (c) propofol product ion scan; (d) thymol product ion scan. The insets in panels (a) and (b) give the structures of the parent compounds propofol and thymol, respectively.

Ionization of propofol was attempted with both TIS (Turbo Ion Spray) as well as APCI sources. Neither ionization technique allowed for detection of propofol in the positive ion mode, because propofol is difficult to protonate. In the negative ion mode propofol produces a stronger signal with APCI than with TIS. This is due to the fact that APCI tends to give better sensitivity for less polar compounds such as propofol [17]. Furthermore, during the LC optimization we observed that the propofol MRM signal (*m/z* 177/161) was much stronger with methanol as the mobile phase compared to acetonitrile and buffer. This finding is expected, because methanol is the most polar of the solvents tested and thus improves ionization. Optimal ionization of propofol was achieved using methanol–water with 0.05% ammonium hydroxide (98:2) at a flow rate of 1 ml/min. Lower flow rates or increases in the aqueous content of the mobile phase decreased the ionization of propofol.

3.3. Limit of detection and linearity

Chromatographic separation adequately separated propofol and the internal standard thymol from interfering plasma constituents not eliminated by SPE. Under the described chromatographic conditions, the retention times for propofol and the internal standard thymol were 3.29 and 3.35 min, respectively. The total run time was 6.0 min. Despite these overlapping retention times, detection by APCI-triple quarupole

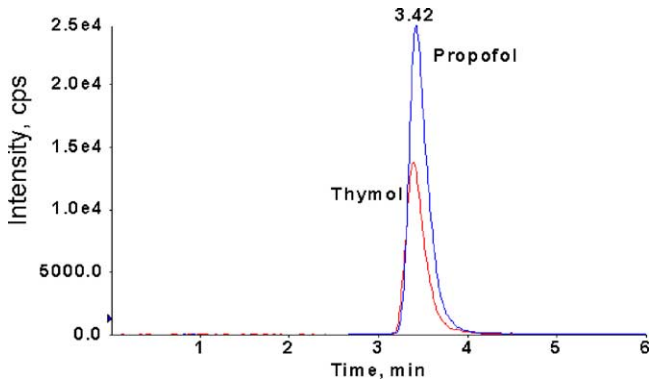

Fig. 4. Plasma sample of a patient under propofol sedation.

MS discriminated propofol and thymol (Fig. 3). Representative total ion chromatograms of propofol-spiked human blank plasma are shown in Fig. 2. The insert shows the extracted ion chromatograms from a run time of 3–4.5 min, thus isolating the propofol peak in a chromatographic plot, which is scaled to maximize the propofol peak.

A linear relationship was obtained between the ratio of the peak area of propofol to that of thymol and the amount of propofol added to plasma in a range of 5–2000 ng/ml. No change in slope was observed with various samples of plasma used. Calibration curves in methanol and plasma almost passed through the origin with correlation coefficients of 0.9998 and 0.9994 for methanol and plasma, respectively. Values of slope and intercept were 0.0010 and 0.00741 for the calibration curve done in methanol whereas they were 0.0010 and 0.0080 for the curve done in plasma. Based on these curves the limit of quantitation of our method is 5 ng/ml. However, as shown in Fig. 2B even a plasma concentration of 1 ng/ml can be resolved with a signal-to-noise ratio >10.

The concentration range of propofol standards was chosen to cover the usual range of concentrations required for pharmacokinetic analysis and a typical sample size of 1 ml. Given that sedative and anesthetic concentrations of propofol in man are 1000–6000 ng/ml [18], adequate quantitation of propofol with our assay during anesthesia would be possible from samples of 0.1 ml or less. Indeed the method has been used successfully in a clinical project comparing plasma propofol concentrations with those detected at end-expiration. An example of the extracted chromatogram of a patient under propofol sedation is provided in Fig. 4.

3.4. Precision and accuracy

The accuracy and precision of the assay are summarized in Tables 2 and 3. To determine the intra-assay accuracy and precision, triplicate analyses of plasma samples with three propofol concentrations (10, 100 and 1000 ng/ml) were performed on the same day (Table 2). The coefficient of variation (CV) for the intra-day precision ranged from 0.6–2.0%. Inter-assay accuracy and precision were determined at the same three concentrations analyzed over a period of 3 days

Table 2
Intra-day variability of the assay

Spiked concentration (ng/ml)	Measured concentration (ng/ml)	CV (%)	Accuracy (%)
10	10.6 ± 0.2	2.0	106.0
100	105.3 ± 2.9	2.7	105.3
1000	1016.7 ± 5.8	0.6	101.6

Data are mean ± S.D. of triplicate determinations repeated three times during 1 day. CV: coefficient of variation.

Table 3
Inter-day variability

Spiked concentration (ng/ml)	Measured concentration (ng/ml)				CV (%)	Accuracy (%)
	Day 1	Day 2	Day 3	Mean ± S.D.		
10	9.2	9.9	10.0	10.2 ± 0.6	5.3	102.0
	10.7	11	9.6			
	10.4	10.5	10.5			
100	102.0	105.0	97.8	100.6 ± 3.3	2.9	100.6
	100.0	105.0	103.0			
	98.7	97.2	96.8			
1000	1040	1000	954	991.7 ± 34.1	2.9	99.2
	960	985	996			
	964	1050	976			

Data are mean ± S.D. of triplicate determinations done from samples extracted on three different days. CV: coefficient of variation.

(Table 3) by preparing the fresh calibration curves in triplicate each day for the analysis. The CV for the inter-day precision ranged from 2.9–5.3%. The accuracies were determined by comparing the mean calculated concentration of the spiked plasma samples with the target concentration. The inter- and intra-assay accuracies for all the samples were found to be within 99–105%.

The reproducibility, accuracy and precision of the LC/MS/MS method described here compare favorably with the other methods of propofol detection, particularly near the limit of quantitation [11,12,15]. Coupling of separation by HPLC to the MS with APCI ionization leads to a very specific and sensitive analytical technique. A second stage of the mass analysis (MS/MS) further enhances specificity and provides an improved signal-to-noise ratio compared with single stage MS. Compared to HPLC methods using UV or other traditional modes of detection, the specificity of the MS/MS detection minimizes the need to resolve the analyte from the endogenous matrix simplifying sample preparation requirements.

4. Conclusion

In summary, our analytic method for propofol is simple and highly reliable with no interfering peaks from plasma. This method avoids the step of concentrating the organic extract and hence loss of propofol through evaporation. The increased specificity, selectivity and sensitivity offered by

selectively monitoring compound-specific masses of the fragment ion with mass spectrometry resulted in advantages over previously reported modes of detection. The method reported in this study advances the analysis of propofol plasma concentrations by combining simple, rapid and efficient SPE of propofol from plasma with specific and sensitive quantitation by HPLC with APCI-MS/MS detection.

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