ORIGINAL ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Spectral Resolving of Propofol and Benzyl Alcohol Mixture

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Received: 21. 09. 2023

Revised: 09. 10. 2023

Accepted: 15. 10. 2023

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Abstract

Two simple spectrophotometric methods are proposed for the spectral resolving of propofol and benzyl alcohol in their synthetic mixture. 1st derivative spectrophotometry (Method I) is used for the determination of propofol at 324.1 nm with no significant interaction from benzyl alcohol, while spectral subtraction followed by 1st derivative spectrophotometry (Method II) is used for the determination of benzyl alcohol at 308.15 nm, successfully eliminating the interference from propofol. Both methods were with acceptable linearity (20-80 µg/mL and 3-12 µg/mL for propofol in Method I and benzyl alcohol in Method II, respectively). The standard deviations were 0.00069 and 0.00293 for method I and II, respectively and the correlation coefficient (r) is equal to 0.9999 for both methods, indicating excellent linearity. The reported methods show promise for future application on their coformulated dosage forms, enabling successful determination of both compounds of interest, which can be useful for routine quality control of monitoring maximum concentration of benzyl alcohol used as a preservative.

Keywords: Propofol: benzyl alcohol: 1st derivative spectroscopy: spectral subtraction

1. Introduction

Propofol, known chemically as 2,6-Diisopropylphenol (fig. 1), is an intravenous sedative hypnotic agent chemically unrelated to other intravenous hypnotic compounds and is characterized by having a rapid onset of action due to its high lipophilicity, enabling it to rapidly cross the blood brain barrier (McKeage and Perry, 2003, Bryson et al., 1995). It is used in the sedation of mechanically ventilated adults in the intensive care unit (McKeage and Perry, 2003). Various methods have been devised for its determination in different matrics such as HPLC (Ayad et al., 2018, Bajpai et al., 2004, Cussonneau et al., 2007, Emara et al.,

1999, Favetta et al., 2000, King et al., 2006, Knibbe et al., 1998, Teshima et al., 2001, Vree et al., 1999, Zhang et al., 1998), UV spectrophotometry (M. et al., 2019), Colorimetry (Gad-Kariem and Abounassif, 2000), Capillary electrophoresis (Hui et al., 2009), fluorescence optical fiber detection (FOFD) technology coupled to an on-line molecularly imprinted polymer solid phase extraction (MIP-SPE) (Li et al., 2012) and sequential injection techniques (Li and Li, 2016, Šrámková et al., 2014).

Benzyl alcohol (fig. 1) is an aromatic alcohol that has multiple uses such as local preservative and antibacterial (Gershanik et al., 1982, Nair, 2001). Different methods have been reported for its analysis, including gas chromatography (Dasgupta and Humphrey, 1998, Pérez-Lamela et al., 1993), UV spectrophotometry (Ghasemi et al., 2005a, Ghasemi et al., 2005b), HPLC (Menon and Norris, 1981, Pérez-Lozano et al., 2005, udina et al., 2005) and Fourier transform infrared (FTIR) spectroscopy (Tarhan et al., 2022).

Benzyl alcohol can be used as an antibacterial agent in various pharmaceutical formulations intended for intravenous administration at concentrations ranging between 0.9% and 2% (Johnson et al., 2017) with the European union restricting its concentration to 1% (Nair, 2001). Several reports document some toxic effects for benzyl alcohol (Evens, 1975, Hetherington and Dooley, 2000, Lopez-Herce et al., 1995, Chang et al., 2008), thus indicating the need for devising a simple inexpensive method for monitoring of benzyl alcohol reaction to comply with the ratios mandated by the different authoritative administrations. This paper reports two simple UV spectrophotometric methods for the simultaneous detection of both propofol and benzyl alcohol in their synthetic mixture.

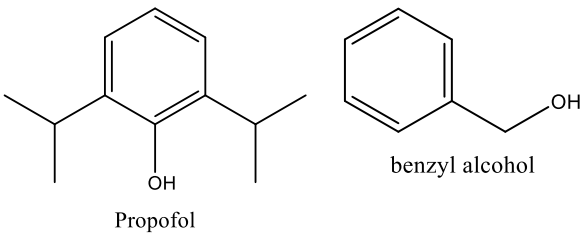

Fig 1. Structures of the studied compounds

2. Experimental

2.1. Apparatus

- Jenway® 6850 Double-Beam Spectrophotometer with Variable Bandwidth, using 1.0 cm quartz cells. Scans were determined in the range of 190-500 nm at 0.2 nm intervals. Prism 5.51 PC software was used for spectrum and data collection. Shimadzu UVprobe 2.42 version was used for derivative spectrum measurement and mathematical manipulation.

- Ultrasonic bath, model SS 101 H 230, USA for sonication.

2.2. Standards

- Propofol with a purity of 99.90% as stated by the manufacturers, were kindly provided by National Organization for drug control and research (NODCAR), Cairo, Egypt.

- Benzyl alcohol with a labelled purity of 99.8% was obtained from ChemCenter.

2.3. Solvents and materials

- All chemicals used during the study were of Analytical Reagent grade and HPLC grade solvents were also used.

- All organic solvents such as methanol, ethanol, and acetonitrile were purchased from Sigma-Aldrich, Germany.

- Deionized double distilled water (Human Resource system, USA)

2.4. Standard stock solutions

Stock standard solutions of propofol, and benzyl alcohol (1000 µg/mL) were separately prepared in 100 mL volumetric flasks by dissolving the pure material in 60 mL of methanol added stepwise, sonicated for 10 minutes and then completing to the volume with methanol. Serial dilutions with methanol were applied for preparation of the standard solutions for prepared mixtures.

2.5. Experimental procedure

2.5.1. Spectral characteristics

Working solution of propofol of concentration 20 µg/mL and of benzyl alcohol of concentration of 3 µg/mL were prepared by transferring appropriate volumes from their corresponding stock solutions to 10mL volumetric flasks and then diluting to the mark with methanol. The blank used was 2 mL methanol in distilled water and 0.3 mL methanol in distilled water for propofol and benzyl alcohol respectively. The zero-order spectra of each compound were measured within the range 190–500 nm and stored in the computer.

2.5.2. Preparation of synthetic mixtures

Four synthetic mixture were prepared with the concentration ratios of 20, 40, 60, 80 µg/mL for propofol and 3, 6, 9, 12 µg/mL for benzyl alcohol for the 1st derivative technique and the spectral subtraction followed by 1st derivative technique.

2.5.3. Application of 1st derivative spectrophotometry

Series of concentrations of propofol (20, 40, 60, 80 µg/mL) were prepared by transferring appropriate volumes from its stock solution in 10 mL volumetric flasks and diluting to the mark with methanol. Each obtained spectrum was smoothed (Δλ= 5 nm) and the 1st derivative spectra was obtained (Δλ= 5 nm, scaling factor = 100). Propofol was determined at wavelength **324.1 nm** and the calibration curve was constructed by plotting the amplitudes at the chosen wavelength versus the corresponding concentration.

2.5.4. Application of spectral subtraction followed by 1st derivative technique

At first, the normalized absorptivity curve of propofol was calculated by dividing the zero order spectra of pure propofol by their corresponding concentrations. Four curves were used to calculate the average absorptivity curve of propofol. Next, after applying the 1st derivative technique, the obtained zero order spectra for propofol were calculated by multiplying the average absorptivity curve with each obtained concentration. Then, the zero order spectra of benzyl alcohol were obtained by subtracting the obtained zero order spectra for propofol from the zero order spectra of the corresponding synthetic mixture. The obtained benzyl alcohol spectra were smoothed ((Δλ= 5 nm) and the 1st derivative spectra was obtained (Δλ= 5 nm, scaling factor = 100).

The wave length **308.15 nm** was chosen for constructing the benzyl alcohol by plotting the amplitudes at the said wavelength versus the corresponding concentrations. 1st derivative spectra for benzyl alcohol were used to overcome the problems of the resultant indefinite peaks in the zero order spectra.

3. Results and discussion

The zero-order absorption spectra of propofol and benzyl alcohol (fig. 2) show severe overlap, hampering the direct determination of both compounds.

Propofol was successfully determined using 1st derivative technique with no interference from benzyl alcohol at wavelength 324.1 nm (fig. 3).

Benzyl alcohol was successfully determined using the 1st derivative technique after spectral subtraction at 308.15 nm (fig. 4).

3.1. Optimization of experimental parameters

3.1.1. Effect of different solvents

Different solvents were utilized such as water, methanol, ethanol, acetonitrile, 0.1N NaOH and 0.1N HCl. Water, 0.1N NaOH and 0.1N HCl couldn't be used since they lead to the precipitation of propofol since it's very slightly soluble in water (Moffat et al., 2011). Methanol was found to give the highest absorptivity in addition to its greenness, so it was chosen as a solvent for this method.

3.2. Analytical performance

The parameters of analytical performance are summarized in **table 1**.

3.2.1. Linearity and range

The linearity of propofol was found to be 20-80 µg/ml (fig. 5) while the linearity of benzyl alcohol was found to be 3-12 µg/ml (fig. 6). The calibration data are summarized in **table 2**.

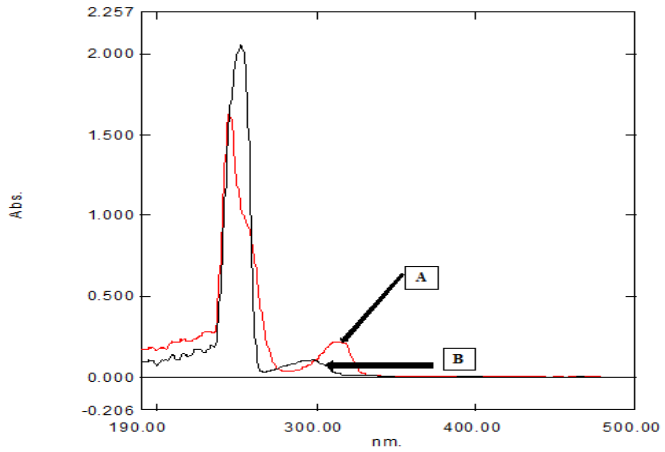

Fig 2. Zero order spectra of propofol (20 µg/mL) (A) and benzyl alcohol (3 µg/mL) (B) in methanol

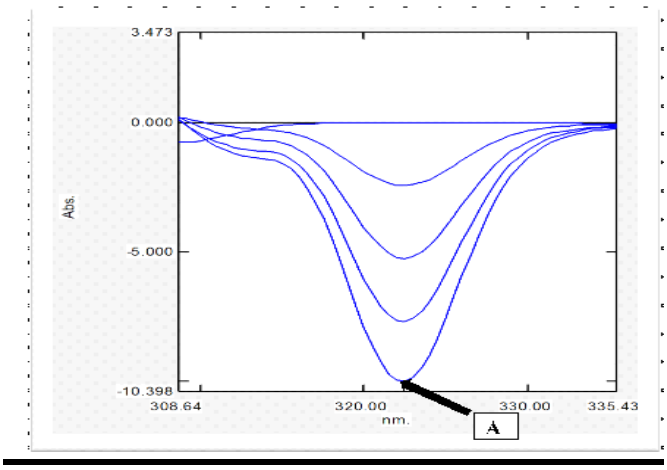

Fig 3. First derivative spectra of propofol (20, 40, 60, 80 µg/mL) indicating zero crossing with benzyl alcohol (3 µg/mL) at 324.1 nm (A).

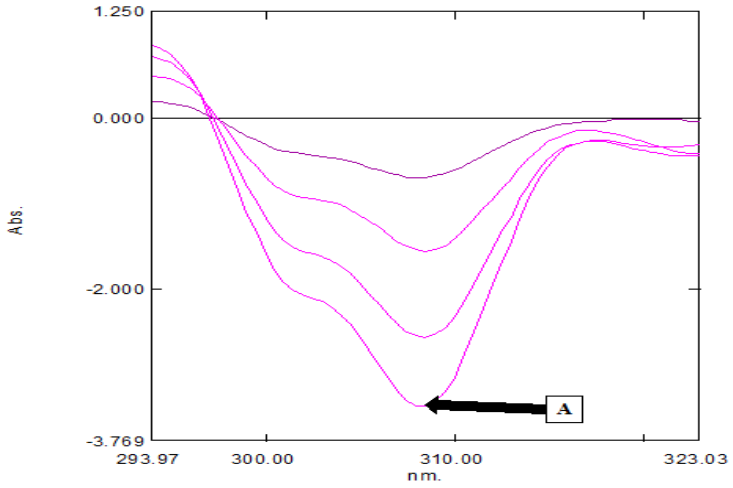

Fig 4. Benzyl alcohol first derivative at 308.15 nm (A) after spectral subtraction.

Table 1: Performance parameters of the analytical methods.

Parameters	Propofol	Benzyl alcohol
	1st derivative technique	1st derivative technique after spectral subtraction
Linearity (µg/ml)	20-80	3-12
LOD (µg/ml)	1.169	0.274
LOQ (µg/ml)	3.542	0.831
Correlation coefficient (r)	-0.9999	-0.9999
Slope	-0.1074	-0.2893
Intercept	-0.1755	0.1820
$S_{y/x}$ (standard deviation of residuals)	0.0311	0.0196
S_a (standard deviation of the intercept of the regression line)	0.038	0.0241
S_b (standard deviation of the slope of the regression line)	0.00069	0.00293
% Error	0.198	0.36
% RSD	0.395	0.719
Mean Found (%) ± SD	99.95 ± 0.395	99.95 ± 0.719

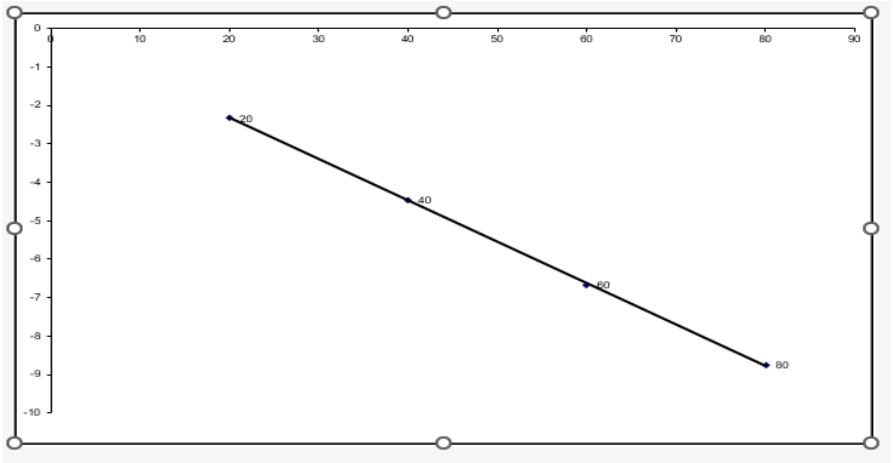

Fig. 5. Calibration curve of propofol.

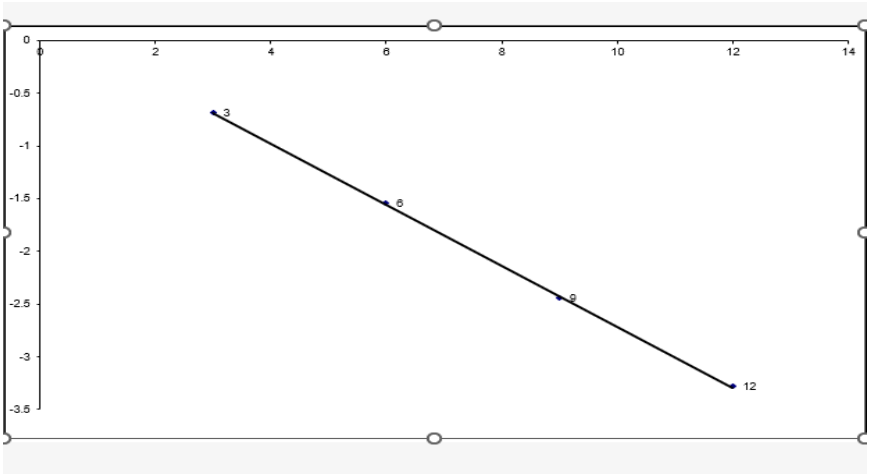

Fig. 6. Calibration curve of benzyl alcohol.

Table 2: Calibration data of propofol and benzyl alcohol

Propofol			Benzyl alcohol		
Conc (µg/ml)	**Response**	**% Recovery**	**Conc (µg/ml)**	**Response**	**% Recovery**
20	-2.318	99.77	3	-0.686	100
40	-4.46	99.74	6	-1.542	99.23
60	-6.6195	100.54	9	-2.444	100.92
80	-8.7675	99.74	12	-3.278	99.65

3.2.2. Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) was found to be 1.169 and 0.274 µg/mL while the limit of quantification (LOQ) was found to be 3.542 and 0.831 µg/mL for propofol and benzyl alcohol, respectively.

3.2.3. Selectivity

Propofol was successfully determined using the 1st derivative technique at 324.1 nm with no significant interference from benzyl alcohol. Furthermore, Interference from propofol was successfully eliminated using spectral subtraction followed by 1st derivative technique and benzyl alcohol was appropriately determined at 308.15 nm, illustrating the selectivity of both techniques for the compounds of interest.

4. Conclusion

Two simple spectrophotometric techniques were proposed for the resolving of propofol/benzyl alcohol mixture. Propofol was successfully determined using 1st derivative technique at 324.1 nm, while benzyl alcohol was determined using spectral subtraction followed by 1st derivative techniques. The proposed methods showed acceptable linearity and selectivity and shows promise to resolve the mixture of interest in commercial products.

Conflict of interest: none declared by the authors.

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