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Short Communication

The determination of 2,6-diisopropylphenol (propofol) in an oil in water emulsion dosage form by high-performance liquid chromatography and by second derivative UV spectroscopy

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Introduction

2,6-Diisopropylphenol (propofol) is an intravenous anaesthetic agent, structurally unrelated to other anaesthetic agents, that is used for both induction and maintenance of anaesthesia during surgical procedures. The drug is only slightly soluble in water, thus necessitating its formulation as an oil in water emulsion. In addition to propofol (10 mg ml^{-1}), the formulation also contains soybean oil (100 mg ml^{-1}), glycerol (22.5 mg ml^{-1}), and egg lecithin (12 mg ml^{-1}), with sodium hydroxide to adjust pH.

Due to the complex matrix of the drug dosage form, the analyst must be certain that the component that is being detected is indeed the active component, propofol, and not one of the excipients. This possibility is of even greater importance in the absence of a suitably pure standard (>99% pure) or a placebo matrix. In the case where the primary method is a HPLC assay, a second, independent assay is useful in ruling out the possibility of a coeluting impurity and in validating the stability-indicating properties of the primary method. Although there are no citations concerning the analysis of propofol in a dosage form matrix, there have been reports in the literature dealing with the quantitative analysis of propofol in plasma [1, 2]. Typical assays used in pharmacokinetic studies usually involve precipitation of the plasma proteins followed by liquid chromatography (LC) and fluorescence or UV detection. The working concentrations are in the ng ml^{-1} range and often require sample concentration or derivatization to increase the sensitivity. Plummer reported a limit of quantitation of about 2 ng ml^{-1} using a C-18 column and fluorescence detection [1]. In Plummer's study, the samples were extracted into cyclohexane and concentrated before being chromatographed. Pullen *et al.* reported an internal surface reversed-phase LC method to separate the plasma matrix from the drug [2]. In this approach, the C-18 material is bonded at the internal pore surfaces of the stationary support material. The small drug molecules diffuse into the pores and interact with the bonded C-18 material, while the plasma macromolecules are excluded from the pores due to their larger molecular size. Thus, propofol is directly separated from plasma using a combination of reversed-phase and size-exclusion chromatography. Direct injection of a complicated matrix may result in clogging of the frit and column, thus requiring frequent maintenance of the HPLC system.

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A simple, accurate method is described in this paper for the quantitative, stability-indicating determination of propofol in its oil in water emulsion dosage form by LC. Also described is the development and validation of a second confirmatory method which employs second derivative UV spectroscopy.

Differential spectrophotometry was first proposed by Giese and French in 1955 as a new approach to the quantitative measurement of the absorption intensity and of the wavelength of maximum absorption for a compound in a multicomponent system [3]. The derivative of a UV spectrum is calculated electronically and presented as $d^2A/d\lambda^n$, where A is the absorbance, λ is the wavelength, and n is the order of the differential. A second derivative UV spectrum is a plot of $d^2A/d\lambda^2$ versus λ, which represents the differential change in the slope of the first derivative UV spectrum ($dA/d\lambda$ versus λ) against the wavelength. This is significant because maxima and minima in the zero-order UV spectra present themselves as "zero crossings" or points at which $d^2A/d\lambda^2$ is equal to zero in the second derivative spectrum, independent of the original magnitude of the maxima or minima. Therefore, judicious selection of a wavelength where the spectrum of the matrix undergoes a zero crossing, but the spectrum of the analyte of interest does not, allows for the quantitative determination of the analyte. Another important characteristic is that sensitivity in this method is dependent upon the rate of change of the molar absorptivity, ϵ, at a given wavelength rather than ϵ itself. Large rate changes will result in sharp, easily quantified peaks. Thus, poorly UV absorbing compounds can be detected and quantified by selecting wavelengths where a large rate change in ϵ occurs.

Experimental

Reagents and materials

Methanol and monobasic ammonium phosphate, HPLC grade, were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The propofol standard that was used throughout the study, 2,6-diisopropylphenol, was obtained from Aldrich Chemicals [Milwaukee, WI, USA (lot No. 01729ET)]. The labelled purity was 97% and the standard was used without further purification. Diprivan Injection single use, 20-ml ampules (10 mg ml^{-1}) (Stuart Pharmaceuticals, Wilmington, DE, USA) was purchased from a local pharmacy. Food-grade soybean oil and soy lecithin were used in the manufacture of a placebo emulsion.

Equipment and apparatus

The pH was set using a digital ionanalyzer/501 from Orion Research Inc. (Cambridge, MA, USA), and a glass combination pH electrode from Fisher Scientific (Fairlawn, NJ, USA). Spectroscopic measurements were performed on an HP-8451A diode array spectrophotometer (Hewlett–Packard, Avondale, PA, USA). The LC system consisted of a Rabbit-HP pump; a Dynamax Microsorb phenyl column (5 μm, 150 × 4.6 mm i.d.) with a matching guard column from Rainin, Inc. (Woburn, MA, USA); an Apple II computer controller from Apple Computer (Cupertino, CA, USA); a Gilson 704 data acquisition system from Gilson International (Middleton, WI, USA); an Isco V4 variable wavelength detector from Isco, Inc. (Lincoln, NE, USA); and an autoinjector from Micromeritics (Norcross, GA, USA). A microcentrifuge, model 235, from Fisher Scientific (Fair Lawn, NJ, USA) was also used in the study. Standard volumetric pipettes and low actinic glassware were used throughout the studies.

Procedures

Standard solutions. Standard solutions were prepared by diluting aliquots of 2,6-diisopropylphenol with methanol to achieve the following concentrations: 0.10, 0.15, 0.20 and 0.25 mg ml^{-1}.

Second derivative UV assay. Samples of propofol injection were diluted to a final concentration of approximately 0.2 mg ml^{-1} with methanol. The samples were then centrifuged at high speed (13 600**g**) for 4 min in a microcentrifuge. The precipitate was discarded, and the supernatant was treated as the final sample solution whose spectrophotometric response was read in the second derivative mode. The measured response $d^2A/d\lambda^2$ of the sample was compared with the measured response of the standard, where A is the absorbance and λ is the wavelength. A wavelength, 286 nm, corresponding to a zero crossing for the matrix was used in this method.

HPLC assay

Samples were diluted with methanol to a final concentration of approximately 0.2 mg

ml^{-1}. The diluted samples were centrifuged in a microcentrifuge at high speed (13 600**g**) for 4 min and the precipitate was discarded. The supernatant was treated as the final sample solution. A 10-μl aliquot of the final sample solution was chromatographed using 276 nm as the detection wavelength. The mobile phase used in the LC method consisted of methanol–monobasic ammonium phosphate aqueous buffer (0.3 M, pH 5.0) (70:30, v/v). Sample peak areas were compared with those of the standard.

Assay validation

Because the reliabilities of the analytical methods were not known, validation was necessary. The parameters that were used to assess the reliability of the methods were precision, accuracy and linearity. A blank emulsion (placebo) containing the same inactive components in the same concentrations as the propofol dosage form was prepared by first mixing the lecithin and the soybean oil together in a blender to form the oil phase. Water was then blended into this mixture to form the emulsion. To this blank emulsion sufficient 2,6-diisopropylphenol was added to achieve a concentration of 10 mg ml^{-1}. Dilutions of the test emulsion were made such that final sample concentrations were 0.10, 0.15, 0.20 and 0.25 mg ml^{-1}. The sample solutions were then analysed in triplicate by both the UV and LC methods.

Results and Discussion

Spectroscopic method

A characteristic second derivative UV spectrum for propofol is shown in Fig. 1 as a solid line. The dotted line shows the second derivative UV spectrum for the matrix obtained from a blank emulsion that was treated in the same manner of preparation as the propofol-containing emulsion. The spectrum obtained for propofol was shifted so that it would be readily compared with the spectrum obtained from the blank emulsion. As expected, the presence of propofol in the dosage form sample gave a spectrum markedly different from that obtained from the matrix. At the analytical wavelength (286 nm) the spectrum for the blank emulsion exhibited a zero crossing, whereas the spectrum for propofol was at a maximum. Therefore, the response observed for the dosage form at 286 nm was attributable solely to propofol, and not to any of the matrix components.

The second derivative UV method for propofol was validated over the propofol concentration range of 0.10–0.25 mg ml^{-1}. Linearity of the method was determined from a standard curve for propofol and is shown in Table 1. A least-squares fit of the standard curve yielded a straight line with a slope of 0.128 and an intercept of 6.68×10^{-3}, and a correlation coefficient of 0.976. A negative deviation from linearity was observed at concentrations

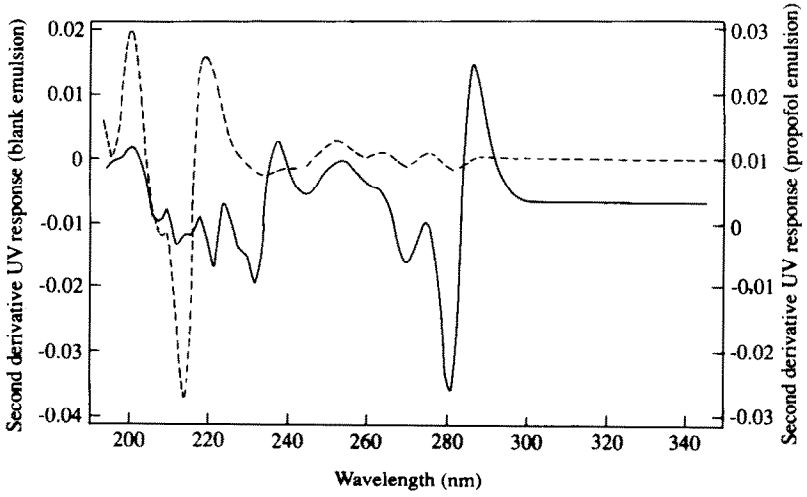

Figure 1
Second derivative UV spectrum for propofol emulsion (solid line) and blank emulsion (dotted line).

Table 1
Standard curve data for propofol from second derivative UV and HPLC methods

Concentration (mg ml^{-1})	Average response	RSD* (%)
	Second derivative UV	
0.10	0.0178	0.162
0.12	0.0220	0.373
0.15	0.0268	0.747
0.16	0.0283	0.260
0.20	0.0336	0.185
0.22	0.0354	0.566
0.24	0.0368	0.466
0.25	0.0374	0.695
0.10	LC	0.583
0.15	48047	1.745
0.20	72047	0.529
0.25	95863	1.256
	120906	

*$n = 6$.

>0.20 mg ml^{-1}. The intercept of the propofol standard curve corresponded to the baseline noise which indicated the response of the analytical method in the absence of the analyte, propofol. The theoretical limit of detection, defined as the concentration that produced a response signal whose magnitude was twice the baseline noise, was determined from the intercept and the slope of the regression equation. The theoretical limit of detection for the second derivative UV method is 0.0521 mg ml^{-1}. It should be noted that the limit of detection is not necessarily correlated to the limit of quantitation since the limit of detection is often not included in the linear range.

Liquid chromatography method

A characteristic chromatogram for propofol is presented as the solid trace in Fig. 2. A chromatogram of the matrix is included as the dotted line in Fig. 2 for comparison. Propofol eluted as a single band with a retention time of about 4 min. It was well resolved from the other components in the matrix that eluted from the column in 1–2 min.

The LC assay was validated for propofol, whose concentration was varied from 0.10 to 0.25 mg ml^{-1}. The standard curve date for propofol is shown in Table 1. A least-squares fit of concentration versus response exhibits linear behaviour over the entire concentration range studied and has a slope of 4.88×10^5 and an intercept of -621.8 with a correlation coefficient of 1.000.

Validations

Table 2 summarizes the data used to determine the precision and accuracy of the two analytical methods. The precision was estimated by the relative standard deviation (RSD) which ranged from 0.36 to 0.60% ($n = 6$) for the second derivative UV method. Accuracy was determined by the per cent recovered by the analytical method from a solution containing a known amount of propofol. The per cent recovered for the second derivative UV method ranged from 81.0 to 111.5%, with the lower recovery being observed for the highest concentration standard, 0.25 mg ml^{-1}. As stated earlier, at this concentration deviation from linearity was ob-

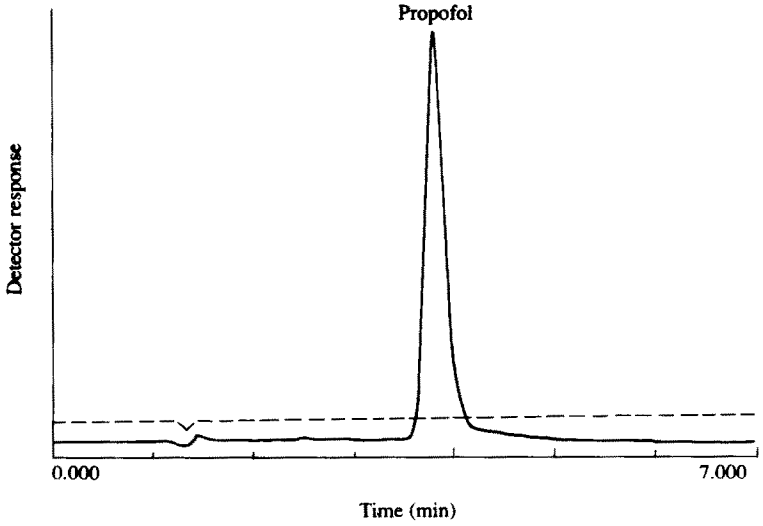

Figure 2
HPLC chromatogram for propofol (solid line) and blank emulsion (dotted line).

Table 2
Validation of HPLC and second derivative UV method

		Second derivative UV			HPLC		
Sample no.	Actual concentration (mg ml^{-1})	Calculated concentration (mg ml^{-1})	RSD* (%)	Recovery* (%)	Calculated concentration (mg ml^{-1})	RSD* (%)	Recovery* (%)
1	0.1000	0.1115	0.54	111.5	0.0989	1.01	98.9
2	0.1500	0.1668	0.6	111.2	0.1598	1.56	106.5
3	0.2000	0.1934	0.36	96.7	0.2011	1.39	100.5
4	0.2500	0.2025	0.44	81.0	0.2592	3.28	103.7

*$n = 6$.

served, a behaviour which may be ascribable to light scattering from small particles of the dosage form emulsion. As the concentration of the absorbing component, propofol increased, the refractive index, arising from the propofol as well as the other non-absorbing components, could be expected to increase. The net result of an increased refractive index would be a negative deviation from linearity. For the LC method the RSD ranged from 1.01 to 3.28% and the per cent recovered ranged from 98.9 to 106.5%.

Figure 3 is a plot of the results obtained by the HPLC method on standard solutions versus those obtained by the second derivative UV method on the same samples. When the point of highest concentration is included, the correlation coefficient is lowered to 0.900. The two methods correlated well with each other at the lower concentrations tested with the correlation coefficient for the regression line that fits the lower three points being >0.99 and the slope being 0.8094. The lack of acceptable correlation between the two methods at high concentrations can be attributed to the deviation from linearity at concentrations above 0.2 mg ml^{-1} for the second derivative UV method.

Conclusions

It was shown that quantitative measures of propofol concentration could be obtained from the oil-in-water emulsion matrix of the dosage form by second derivative UV spectroscopy or LC. The second derivative UV method exhibited a negative deviation from Beer's law at concentrations >0.2 mg ml^{-1}. In contrast, the LC method showed linear behaviour throughout the entire concentration range (0.10–0.25 mg ml^{-1}) studied. Both methods exhibited good reproducibility with the LC method showing a RSD of 0.36–0.60%, while the range of the second derivative method was 1.01–3.28%. In terms of accuracy, the LC method was more accurate than the second derivative UV method based on data obtained from analysis of an extemporaneously prepared placebo which was spiked with known amounts of analyte. Either of the developed

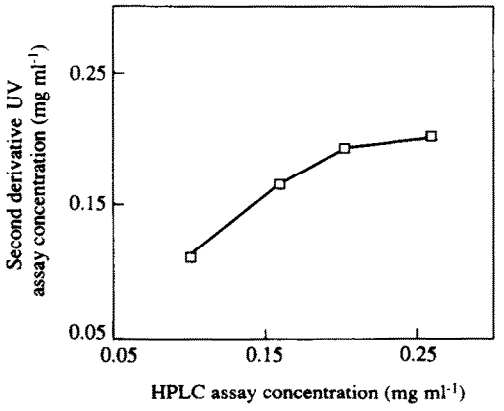

Figure 3
Correlation between the HPLC method and the second derivative UV method.

methods has been shown to provide simple, accurate measurement of the active ingredient in the complex dosage form matrix. The LC method is advantageous due to its greater range of accuracy and its ability to be automated; however, the second derivative UV procedure has the advantage of simplicity and speed. More importantly, it has been shown that, in the absence of a reference standard or a placebo dosage form, zero-crossing second derivative UV spectroscopy can be used as a reliable, independent validation procedure to verify the stability-indicating nature of an HPLC assay within the accuracy of the least accurate method.

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