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Propofol assay in biological fluids in pregnant women[1]

Gaetano Ragno [a], Ettore Cicinelli [b], Sergio Schonauer [b], Claudio Vetuschi [a,*]

[a] *Pharmaco-Chemistry Department of University, via E. Orabona 4, 70126 Bari, Italy*
[b] *Obstetrics and Gynaecology Department of University, P.zza G. Cesare, 70124 Bari, Italy*

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Abstract

A rapid, accurate and sensitive UV derivative method was described for measuring the Propofol concentration in some biological fluids. Furthermore two alternative procedures, a gaschromatographic and a colorimetric, were also defined, and the results of the three methods, when applied on blood samples spiked with known amounts of analyte, were compared. The samples were preliminary purified by a solid phase extraction on octadecyl C18 cartridge. The UV derivative method was applied to a pharmacokinetic study on pregnant women undergoing cesarean sections. After an induction dose administration of 2.5 mg kg^{-1}, the maternal and the umbilical vein blood were found to have comparable concentrations of propofol, with a mean half life of about 3.5 min; on the contrary no detectable levels of the drug were found in amniotic fluid. The drug recoveries were $>98\%$ and the response was linear over the range 0.05–40 μg ml^{-1}. © 1997 Elsevier Science B.V.

Keywords: Propofol; Cesarean part; Derivative spectrophotometry; Gaschromatography; Solid phase extraction; Pharmacokinetics; Transplacental passage

1. Introduction

Propofol, 2,6-diisopropylphenol, (PPF) is a recently introduced intravenous anesthetic which produces rapidly anesthesia without exciting effects over the anesthesia phases [1–4] and, actually, is one of the few drugs used in the T.I.V.A. technique (Totally Intra Venous Anesthesia) [5,6]. Since the drug does not show cumulative effects, the anesthesia can be prolonged by repeated injections or continuous infusion [7]. In fact this drug is rapidly distributed in the body and eliminated as a result of a very rapid metabolism [8,9]. The drug is eliminated in the urine as metabolites for 88% and only for 0.3% as parent molecule [1,8].

Pharmacokinetic studies showed that anesthetic effects in man were obtained with a plasma concentration between 1 and 10 μg ml^{-1} [9–11]. The anesthesia induction is obtained with an initial bolus of 2.5 mg kg^{-1} followed by doses between 25 and 50% of the induction dose [12].

The very low solubility of PPF in water and its instability in aqueous solution has made necessary that the drug is formulated as an oil-in-water emulsion (Diprivan®, ICI, Italy) [7,13].

* Corresponding author.

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Table 1
Calibration graphs for PPF (μg ml^{-1}) determination

Method	Signal	Slope	Intercept	r	Variance s^2
Second derivative	$^2D_{285,278}$	15.620 (0.631)	−0.0522 (0.0422)	0.9997	0.0784
Fourth derivative	$^4D_{278,286}$	1041.775 (29.321)	0.0441 (0.0296)	0.9983	0.0400
Gaschromatography	$A_{PRP}/A_{I.S.}$	11.718 (0.473)	−0.136 (0.173)	0.9988	0.533
Colorimetry	A_{610}	6.0260 (0.557)	0.103 (0.228)	0.9975	0.348

In parentheses are reported the confidence intervals; significance level, $P = 0.05$.

Several methods of PPF analysis in biological fluids involved precipitation of the plasma proteins followed by HPLC with UV or electrochemical detection [14–16]. A good increase of the sensitivity has been obtained through drug derivatization with 2,6-dichloroquinone-4-chloroimide (Gibbs' reagent) [17]. In a successive method [18], the drug was directly isolated from plasma using a combination of RP-HPLC and size exclusion chromatography, using an internal surface reversed phase (ISRP). Other chromatographic procedures, HPLC with pre-column extraction [19,20] and GC methods [21,22], have been described. The drug has been also directly assayed in the blood by fluorometry [23], but the sensitivity of this method resulted to be too low to reveal the drug concentration levels occurring in human fluids after therapeutic doses. All these methods are complex and time consuming, with the risk of increasing the drug metabolism during manipulation of the sample.

The present paper proposed a rapid, accurate and sensitive assay method of PPF in human blood and amniotic fluid by second- and fourth-order derivative spectrophotometry. The samples were preliminary purified by a solid phase extraction on Sep-Pak octadecyl C18 cartridge (PPF elution with ethanol). The method has been validated and compared with a referee colorimetric method [17], and with a new gaschromatographic procedure.

The described derivative spectrophotometric method has been applied to a investigation on the placental transfer of the drug to the fetus when it is administered to pregnant women undergoing cesarean sections. In literature the transplacental across of propofol has been demonstrated on laboratory animals, without any teratogenic effect [24].

PPF concentration levels were measured at various times on maternal blood, at birth on fetal blood and also on amniotic fluid, drawn out before opening the amniotic sac. The pharmacokinetic study has been applied on a limited number of cases (six women). Nevertheless the analysis on these samples gave mean results characterized by a good index of precision (RSD < 2.0).

2. Experimental

2.1. Materials

2,6-diisopropylphenol; acetanilide; 2,6-dichloroquinone-4-chloroimide were purchased from Aldrich Chemical (USA); all solvents, supplied by C. Erba (Italy), were of analytical grade.

Sep-Pak C18 cartridges were marketed by Millipore (USA); before use the cartridges were activated by washing with methanol 10 ml and citrate buffer 5 ml.

Gibbs' reagent: isopropanolic solution of 2,6-dichloroquinone-4-chloroimide (1 mg ml^{-1}), prepared just before use.

TMA: mixture of tetramethylammonium hydroxide in methanol (24% p/v) and isopropanol (1:9).

Citrate buffer pH 4.6: citric acid 1.5% and sodium citrate 3% in water.

Pharmaceutical form: Diprivan® emulsion oil-in-water 5% (ICI, Italy).

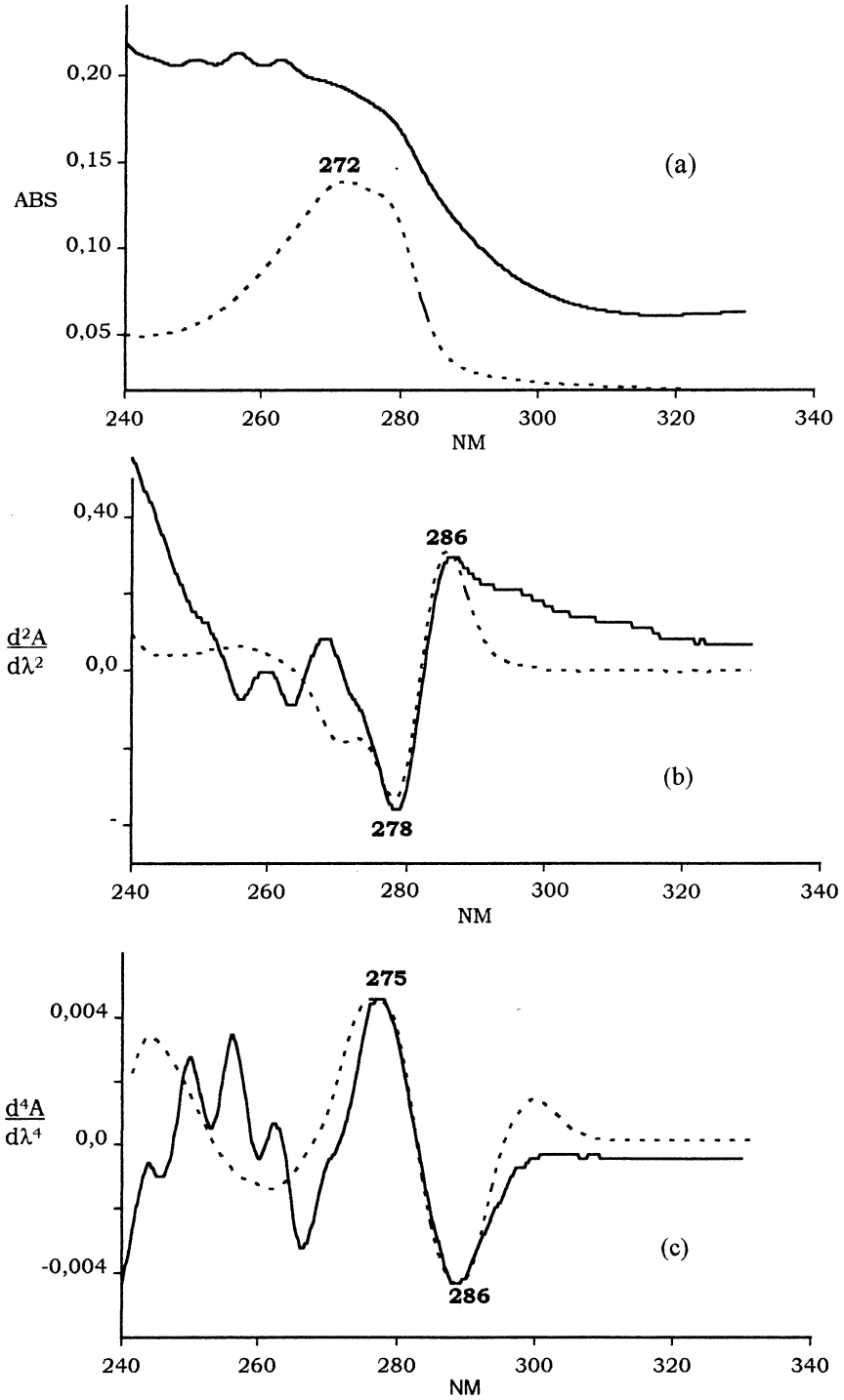

Fig. 1. Absorbance (a), second order (b) and fourth order (c) derivative spectra of PPF (– – –) (8.64 μg ml^{-1}) in and of a cartridge eluate (——) from a blood sample spiked with the same amount of PPF.

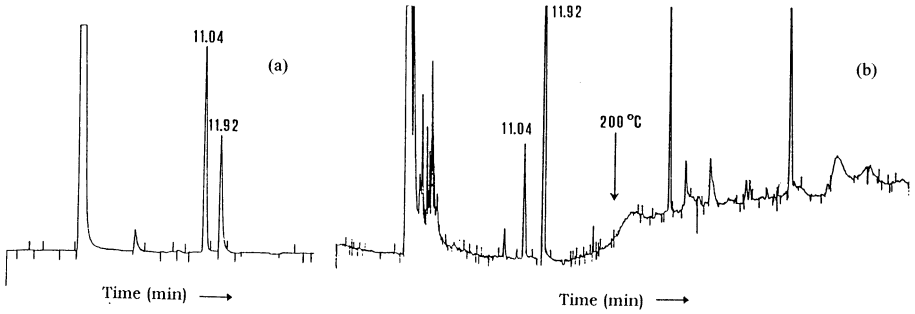

Fig. 2. Chromatograms of a standard solution (a) with PPF 11.52 μg ml^{-1} (Rf 11.04) and internal standard acetanilide (Rf 11.92) and of a cartridge eluate (b) from a blood sample spiked with PPF 5.76 μg ml^{-1}.

2.2. Apparatus

Spectrophotometry: the spectra were recorded over the wavelength range 800–200 for absorbance mode and 330–240 nm for derivative mode, in 10 mm silica quartz cells using a Perkin-Elmer Lambda 16 spectrophotometer; scan speed 2 nm s^{-1}; response (time constant) 1 s; spectral bandwidth and $\Delta\lambda$ were 1 and 6 nm for second-order derivative; 5 and 10 nm for fourth-order derivative, respectively. The spectra were elaborated with software PECSS 4.0 by Perkin-Elmer.

Gaschromatography: the analyses were performed with a Hewlett-Packard Model 5890 Series II gaschromatograph, equipped with a flame ionization detector, using a methyl silicone column 30 m × 0.53 mm × 2.65 μm film thickness (HP-1 by Hewlett-Packard). Operating temperatures were: injector 250°C; detector 300°C; oven 80°C for 16 min rising (20°C min^{-1}) to 200°C. The carrier gas was nitrogen at flow-rate of 20 ml min^{-1}. Injection volume was 1 μl. Data were processed with software GC chemstation A.03.03 by Hewlett-Packard.

2.3. Standard solutions

Twelve standard solutions of PPF were prepared in absolute ethanol with drug concentration varying between 0.01 and 40.00 μg ml^{-1}. For GC analysis an analogous number of solutions were prepared using as solvent ethanol containing the internal standard acetanilide at a concentration of 10 μg ml^{-1}. All injections were 1 μl. These solutions were analyzed to obtain the relationships of instrumental signals versus analytical concentrations.

2.4. Biological samples

Maternal blood samples were drawn out before induction of anesthesia and at approximately 2, 4, 6, 8, 15 and 30 min after administration of the drug. Fetal blood was drawn out at birth from the umbilical vein. Blood samples were collected in vacutainers with sodium citrate and cooled to 4°C to await analysis. Amniotic liquor was collected at birth before opening the amniotic sac.

2.5. Laboratory solutions

Eight synthetic solutions of PPF were prepared adding known amounts of an emulsion vial (PPF 0.50 mg ml^{-1}) to 1 ml of blank blood, in sodium citrate, to obtain reference samples with drug concentration ranging from 0.05 to 20 μg ml^{-1}.

These solutions were used to measure the recovery values.

2.6. Sample preparation

The sample obtained by diluting 1 ml of whole blood with 1 ml of citrate buffer pH 4.6 was flushed (10 drops min^{-1}) on a Sep-Pak C18 cartridge. The cartridge was purged with 30 ml of water then eluted (10 drops min^{-1}) with

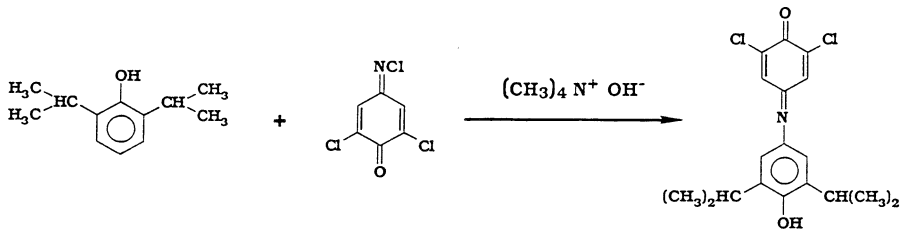

Fig. 3. Derivatization of PPF with the Gibbs' reagent.

absolute ethanol to 5 ml. The eluate was submitted to analysis. The amniotic fluid was directly applied on the cartridge and treated as above described.

2.7. Derivatization with Gibbs' reagent

The procedure was defined in accordance with the method of Adam et al. [17]. Of the sample, 2 ml, were added of 60 μl of Gibbs' reagent and alkalinized with 50 μl of TMA. This solution was shaken and, after 5 min, analyzed.

3. Results and discussion

The absorbance spectrum of Propofol in ethanolic solution presents a maximum at 272 nm. In order to use this signal, not visible in the untreated samples spectra, a simplification of the samples with a solid phase extraction procedure (SPE) was performed. Octadecyl C18 cartridges were loaded with whole biological samples and, after repeated water washing, eluted with absolute ethanol.

Fig. 1 shows the spectra of a PPF standard solution and of a cartridge eluate from a blood sample spiked with the same amount of the drug. Clearly the eluate spectrum resulted not be useful for the drug assay due to the complexity of the matrix.

The derivative spectra in second-order and in fourth-order, on the contrary, presented respectively the specific signals 286–278 and 275–286 nm, whose amplitudes were found to be proportional to PPF concentration. The regression equations obtained are listed in Table 1.

A new gaschromatographic procedure, suitable also as alternative analytical method, was developed to convalide the spectrophotometric results. At above reported conditions, PPF and internal standard acetanilide presented retention times of 11.04 ± 0.35 and 11.92 ± 0.21 min, respectively. Fig. 2 shows a chromatogram of an ethanolic cartridge eluate from a blood sample spiked with a known amount of PPF. The good resolution of the two peaks is evident without any interference of the matrix components. The peak-area ratios between analyte and internal standard of the standard solutions were plotted versus the drug concentrations, giving the regression equation reported in Table 1.

The spectrophotometric and gaschromatographic results were compared with a colorimetric procedure, as a modification of the Adam method [17], using the 2,6-dichloroquinone-4-chloroimide (Gibbs' reagent) as a coupling agent (Fig. 3).

The reaction product, intensively blu-coloured, presented a maximum at 580 nm (Fig. 4) and was not influenced by other components when the procedure was applied to biological samples. The relationship correlating the standard absorbance values and the drug concentrations was reported in Table 1.

The reported derivative method was applied to the PPF assay in blood and amniotic fluid of women undergoing cesarean sections. Anesthesia was induced in all patients with an i.v. dose of 2.5 mg kg^{-1} of propofol.

Table 2 reports the drug concentrations found at sequential times drug administration, carried out with the second derivative spectrophotometric method. None of the differences between

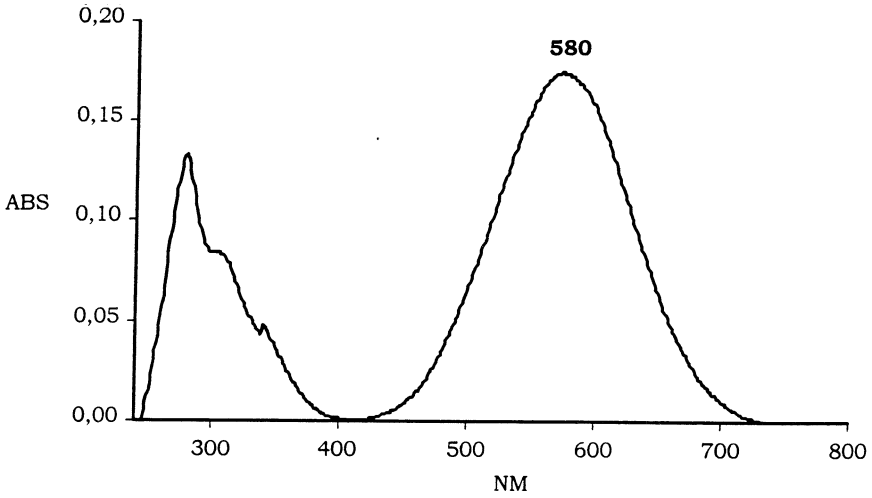

Fig. 4. Absorbance spectrum of the reaction product (1.24 μg ml^{-1}) from coupling of PPF with the Gibbs' reagent.

the series was statistically significant. The graph of Fig. 5 shows the means of PPF concentrations found at the various times with the relative standard deviation. Drug concentrations in all patients declined rapidly for approximately 10 min following the end of dosing. The mean half life resulted to be approximately 3.5 min, in according with the declared very fast metabolism of the drug.

PPF assays were then performed on the blood of the umbilical vein. On each newborn, one sample was drawn out at birth at a time varying from 18 to 26 min after the drug administration. The drug concentration values were found ranging from 0.20 to 0.38 μg ml^{-1}. These concentration values were comparable to the corresponding ones, at the similar times, in the maternal blood, according with a rapid passage of the drug across the placenta [25].

Drug assay was performed also on the amniotic fluid, obtained just before opening the amniotic sac. In all the cases no detectable levels of Propofol were found except one case in which the drug was detected by GC method as traces, of uncertain source. The absence of the drug is in accordance with an its very rapid elimination from the fetal blood circle.

Table 2
Assay of PPF (μg ml^{-1}), calculated on biological samples by second derivative spectrophotometric method

Maternal blood		Fetal blood	
Time (min)	Found (± RSD)	Time (min)	Found
2	4.790 (± 0.0899)	18	0.303
4	2.699 (± 0.1130)	20	0.246
6	1.458 (± 0.0770)	24	0.191
8	0.958 (± 0.0713)	28	0.178
15	0.354 (± 0.0898)	16	0.389
30	0.103 (± 0.0950)	20	0.288

For the maternal blood, PPF concentrations are means (six women) at sequential times after drug administration.
For the fetal blood, PPF concentrations are the values at birth on the umbilical vein at the reported times after administration.

4. Validation

The linearity of response between PPF concentration and the described analytical measures were assured for UV and GC analysis, in a concentration range of drug between 0.05 and 40 μg ml^{-1}, by a correlation coefficient in all cases over 0.99.

Accuracy of the methods were carried out by analysis of blank serum samples spiked with known amounts of PPF over the range 0.05 – 30.00 μg ml^{-1}. The results, shown in Table 3,

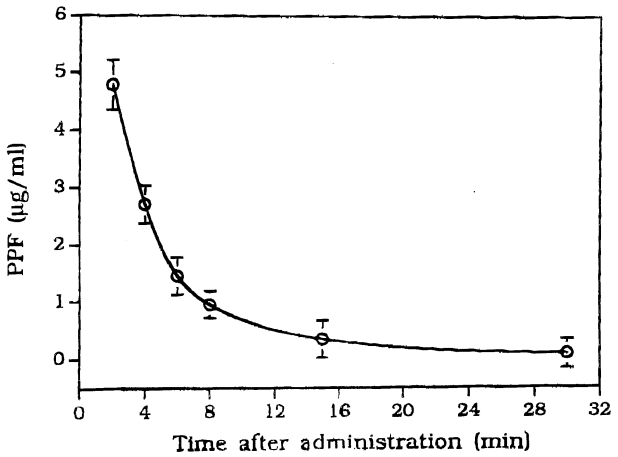

Fig. 5. Means of PPF concentrations (± RSD) measured on blood samples ($n = 6$) of women undergoing cesarean sections after a dose of 2.5 mg kg^{-1}.

demonstrated good recovery (± RSD) with mean values of 99.27 ± 1.86 and 101.55 ± 2.00 for the second- and fourth-order derivative methods, respectively, and a value of 97.29 ± 3.25 for the gaschromatographic procedure. These values were in good accordance with the colorimetric method results, presenting a mean recovery value of 97.27 ± 2.38.

Determination limit for the derivative methods was calculated to be 0.05 and 0.06 µg ml^{-1} for second and fourth order derivative mode. For the gaschromatographic method and colorimetric method the d.l. resulted to be 0.03 and 0.04 µg ml^{-1}, respectively.

Acknowledgements

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Table 3
Assay of PPF in synthetic samples

Spiked concentration (µg ml^{-1})	Analytical method			
	Second derivative	Fourth derivative	Gaschromatography	Colorimetry
0.080	0.078 (1.56)	0.086 (3.35)	0.076 (2.56)	0.082 (2.58)
0.32	0.34 (1.68)	0.35 (1.65)	0.29 (1.95)	0.29 (1.72)
0.96	0.92 (2.32)	0.88 (4.15)	1.02 (3.42)	0.95 (3.23)
2.88	2.95 (1.25)	2.62 (3.68)	2.83 (2.56)	2.45 (2.46)
5.76	5.93 (2.02)	6.56 (3.35)	5.54 (3.13)	5.92 (2.43)
8.64	7.89 (1.09)	7.96 (2.35)	8.35 (1.85)	8.25 (1.82)
11.52	11.02 (2.64)	11.36 (3.08)	10.73 (2.81)	12.21 (2.71)
23.04	23.55 (2.31)	24.95 (4.42)	23.55 (2.06)	22.31 (2.09)

The PPF concentrations found are means of three determinations.
Values in parentheses are RSD%.

References

[1] B. Kay and G. Rolly, Acta Anaesthesiol. Belg., 28 (1977) 303–316.
[2] N.H. Kay, J. Uppington, J.W. Sear and M.C. Allen, Br. J. Anaesth., 57 (1985) 736–741.
[3] K.M. Rogers, K.M.S. Dewar, T.D. McCubbin and A.A. Spence, Br. J. Anaesth., 52 (1985) 807–812.
[4] D.V. Rutter, M. Morgan, J. Lumley and R. Owen, Anaesthesia, 35 (1980) 1168–1172.
[5] T.M. Savag, M.A.E. Ramsay, J.P.J. Curran, J. Cotter, P.T. Walling and B.R. Simpson, Anaesthesia, 30 (1975) 757–781.
[6] M.M. Ghoneim and K. Korttila, Clin. Pharmacokin., 2 (1977) 344–348.
[7] G.C. Cummings, J. Dixon, N.H. Kay, W.P.J. Windsor, E. Major, M. Morgan, J. Sear, A.A. Spence and D.K. Stephenson, Anaesthesia, 39 (1984) 1168–1173.
[8] P.J. Simons, I.D. Cockshot, E.J. Douglas, E.A. Gordon, K. Hopkins and M. Rowland, Postgrad. Med. J., 61 (1985) 64–69.
[9] H.K. Adam, L.P. Briggs, M. Bahar, E.J. Douglas and J.W. Dundee, Br. J. Anaesth., 55 (1983) 97–102.
[10] H.K. Adam, B. Kay and E.J. Douglas, Anaesthesia, 37 (1982) 536–540.
[11] N.H. Kay, J.W. Sear, J. Uppington, I.D. Cockshott and E.J. Douglas, Br. J. Anaesth., 58 (1986) 1075–1079.
[12] I.D. Cockshott, L.P. Briggs, E.J. Douglas and M. White, Br. J. Anaesth., 59 (1987) 1103–1110.
[13] L.C. Bailey, K.T. Tang and B.A. Rogozinski, J. Pharm. Biomed. Anal., 9 (1991) 501–506.
[14] T.B. Vree, A.M. Baars and P.M.R.M. De Grood, J. Chromatogr., 417 (1987) 458–464.
[15] R.A. Vebel, C.A. Wimm, A.O. Hawtrey and J. Coetzee, J. Chromatogr., 526 (1990) 293–295.
[16] G. Mazzi and M. Schinella, J. Chromatogr., 528 (1990) 537–541.
[17] H.K. Adam, E.J. Douglas, G.F. Plummer and M.B. Cosgrove, J. Chromatogr., 223 (1981) 232–237.
[18] R.H. Pullen, C.M. Kennedy and M.A. Curtis, J. Chromatogr., 434 (1988) 271–277.
[19] P. Altmayer, U. Buech, H.P. Buech and R.L. Larsen, J. Chromatogr. Biomed. Appl., 612 (1993) 326–330.
[20] J. Guitton, C.S. Degoute, D. Durand, A.R. Rochette and M. Mauchon, J. Pharm. Clin., 11 (1992) 221–224.
[21] Yu Hsin Ying and Lian Jiun Kai, J. Chromatogr. Biomed. Appl., 615 (1993) 77–81.
[22] P.L. Stetson, E.F. Domino and J.R. Sneyd, J. Chromatogr. Biomed. Appl., 620 (1993) 260–267.
[23] H.K. Adam, J.B. Glen and P.A. Hoyle, Br. J. Anaesth., 52 (1980) 743–748.
[24] J.B. Glen, Br. J. Anaest., 52 (1980) 731–742.
[25] Giu Tony, Yau Gordon, Jong Winston, Tan Perpetua, Leung K.W. and Chan Kelvin, Br. J. Anaesth., 67 (1991) 49–53.

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