

Simultaneous determination of isosorbide dinitrate, midazolam and noradrenaline in isotonic saline solution by UV spectrophotometry and partial least square regression analysis

Alexandre Secq¹, MSc; Damien Lannoy², PharmD; Sophie Dewulf¹, PharmD; Christine Barthélémy², PhD; Bertrand Décaudin^{2,3}, PhD; Professor Pascal Odou^{2,3}, PhD

ABSTRACT

Simultaneous spectrophotometric determination using partial least square regression has been successfully applied to multicomponent analysis of complex mixtures. This work aims at improving a multivariate method on UV spectra to simultaneously determine three drugs commonly used in anaesthesia (isosorbide dinitrate, midazolam and noradrenaline) in order to obtain a dynamic dosage at the end of the extension set. Determinations were made over the concentration ranges of 5-60, 10-80 and 2.5-20 µg/mL for isosorbide dinitrate, midazolam and noradrenaline respectively, in binary mixtures and 6.67-30, 0.83-7.5 and 1.67-23.33 µg/mL for isosorbide dinitrate, midazolam and noradrenaline, respectively, in ternary mixtures. The 220-300 nm spectral zone was the best model with the highest Q²cum index. This method shows limits of detection quite similar to specific high-performance liquid chromatography (HPLC) methods. The recovery study, performed on prediction sets containing eight different ternary mixtures of isosorbide dinitrate, midazolam and noradrenaline, showed recovery percentages in the 99.5-101% range.

KEYWORDS

Isosorbide dinitrate, midazolam, noradrenaline, partial least square, quantification, spectrophotometry

INTRODUCTION

Over the past few years, considerable advances in infusion practices in anaesthesia have resulted from improvements both in therapeutics, such as new drugs with short half-lives, and improvements in technology such as target-controlled infusion systems [1-8]. These

new concepts are based on clinical pharmacokinetic and pharmacodynamic data, the final goal being to infuse the total dose into the patient under appropriate conditions (right dose, right time). A successful procedure depends on optimising devices like the infuser or extension set.

Contact for correspondence: Professor Pascal Odou
Laboratoire de Biopharmacie
Pharmacie Galénique et Hospitalière (EA 4034 – IFR114)
Faculté des Sciences Pharmaceutiques et Biologiques
3 Rue du Professeur Laguesse
BP 83, F-59006 Lille Cedex, France
Tel: +33 3 20 96 40 29
Fax: +33 3 20 95 90 09
pascal.odou@univ-lille2.fr

A sensitive, fast and simple dosage technique is required to compare drug flow rate at the end of the extension set where the angiocatheter enters the patient's vein when drugs are infused altogether, but using different access points. This technique must allow for giving multiple drugs used in anaesthesia practice so as to analyse the impact of multi-infusion.

To perform such a study, three drugs which are widely used in anaesthesia and in intensive care units were selected: isosorbide dinitrate, midazolam and noradrenaline.

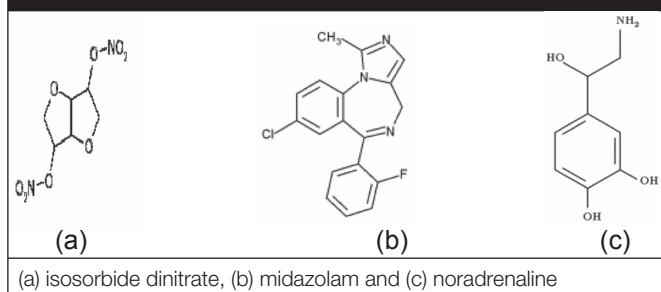
Isosorbide dinitrate (4, 8-dinitrooxy-2, 6-dioxabicyclo[3.3.0]octane) (see Figure 1a), works by relaxing blood vessels and increasing the supply of blood and oxygen to the heart while reducing its work load. This drug is usually used to treat the symptoms of angina (chest pain). In anaesthesia, isosorbide dinitrate is used for perioperative blood pressure management.

¹ Pharmacie, Centre Hospitalier de Dunkerque, Dunkerque Cedex, France

² Laboratoire de Biopharmacie, Pharmacie Galénique et Hospitalière, Faculté des Sciences Pharmaceutiques et Biologiques, Lille Cedex, France

³ Pharmacie Centrale, Centre Hospitalier Régional Universitaire de Lille, Lille Cedex, France

Figure 1: Chemical structure of the drugs analysed



Different methods have been described for its determination in serum and in pharmaceutical products. Isosorbide dinitrate can be quantified by HPLC [9-13], gas-liquid chromatography [14], polarography [15], capillary gas chromatography with electron-capture detection [16] and infrared spectrometry [17].

Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo(1,5-a)(1,4)benzodiazepine) (see Figure 1b), is a hypnotic benzodiazepine used for sedation in critical care and anaesthesia. Midazolam can be quantified in serum and in pharmaceutical products by gas chromatography [18], liquid chromatography [18], gas chromatography-mass spectrometry [18, 19], HPLC [20] and liquid chromatography/electrospray mass spectrometry [21].

Noradrenaline(4-(2-amino-1-hydroxy-ethyl)benzene-1,2-diol) (see Figure 1c), acts on both alpha-1 adrenergic receptors to cause vasoconstriction and beta-1 adrenergic receptors to increase heart rate, stroke volume and cardiac output.

The reference method remains HPLC because it can simultaneously determine catecholamines [22].

As far as is known there has been no previous report of simultaneous spectrophotometric determination of isosorbide dinitrate, midazolam and noradrenaline in solution. Therefore a method based on UV spectrophotometry, using partial least square (PLS) regression was developed. PLS regression is a simple and powerful multivariate method based on factor analysis and is used for building regression models based on latent variable decomposition relating a block of independent variables, x (spectra), to a block of dependent ones, y (concentrations) [23]. When the regression is performed for each independent variable individually, the method is called PLS1. When all independent variables are predicted simultaneously (Y is a matrix, with the same number of columns as analytes), the method is called PLS2.

This method has been successfully applied to the multi-component analysis of complex mixtures [24-27].

The aim of this work was to develop a multivariate method on UV spectra to determine the three drugs simultaneously so that dynamic dosage can be implemented at the point where the drugs enter the patient from the angiocatheter. In practice, this type of study needs thousands of samples and a sensitive and fast method to describe physical characteristics accurately.

METHOD

Chemicals and reagents

IV drugs that were diluted from commercial products: isosorbide dinitrate (Risordan, sanofi-aventis, France), midazolam (midazolam, Dakota Pharm, France) and noradrenaline (noradrenaline, Merck, France) were used. The drugs were not subjected to further purification. For midazolam and noradrenaline, the commercial drugs contain only water, sodium hydroxide or hydrochloric acid. For isosorbide dinitrate, the commercial drug contains glycine, acetic acid and mannitol. An isotonic saline solution (sodium chloride 0.9%, Maco Pharma, France) was used to prepare dilutions of samples according to the usual clinical protocol. Sodium chloride 0.9% also served as the blank during the calibration step.

Apparatus and software

A UV-visible spectrophotometer model (Lamba-25, PerkinElmer, France) combined with 10 mm quartz cells was used to make absorbance measurements. The UV spectra of mixtures were recorded over 200-400 nm wavelengths and absorbance was sampled at 0.5 nm intervals. Spectrum bandwidth was 1 nm, scan speed was 480 nm/min.

All information from the spectrophotometer was collected with UV WinLab software (PerkinElmer, France). PLS regression was performed using a PLS module of XStat software (Addinsoft, USA).

Stock and standard solutions

Stock solutions of isosorbide dinitrate (100 $\mu\text{g/mL}$), midazolam (25 $\mu\text{g/mL}$) and noradrenaline (80 $\mu\text{g/mL}$) were prepared by dissolving the appropriate amount of these compounds from the commercial drugs in isotonic saline solution. Stock solutions were prepared daily from a new vial of drug and working solutions were prepared by adequate dilution of stock solutions in optimum conditions. Ten standard solutions of each drug were prepared to determine the optimal interval of absorbance with a good linearity between absorbance and concentration, according to Beer-Lambert Law and spectrophotometer specifications.

Sample preparation

The contents of each vial were accurately transferred to a 100 mL flask and then the appropriate proportion of sodium

chloride 0.9% was added as diluent. Binary mixtures were prepared by diluting 950 μL of each drug with the appropriate sample. Ternary mixtures were prepared by diluting 500 μL of each drug with the appropriate sample.

Multi-infusion devices

The multi-access devices used in the application are made with polyethylene and polypropylene. They comprise three different access points: proximal and median access (corresponding to a dead volume of 8.01 mL to distal extremity) and distal access (corresponding to a dead volume of 6.39 mL to distal extremity).

PLS analysis

A simultaneous prediction model (PLS) of binary and ternary mixture solutions of isosorbide dinitrate, midazolam and noradrenaline was set up in a mixture solution.

The optimum number of factors used in the PLS-2 algorithm is an important parameter towards obtaining the best prediction performance. This means modelling the system with the optimum amount of information and avoiding overfitting or underfitting. (Overfitting and underfitting means that the defined model must neither overestimate nor underestimate values, and is made by fitting constants). Segmented cross-validation procedures were applied, which consisted of systematically removing a group of the test samples in turn and using only the remaining ones to construct the latent factors and regression. The predicted concentration was then compared with the real one for each of the calibration samples and the predicted error sum of squares (PRESS) was calculated. PRESS was computed in the same manner, and each time a new factor was added to the PLS model. The formula for PRESS is:

$$\text{PRESS} = \sum_{j=1}^n (C_{pj} - C_j)^2$$

where n is the total number of calibration samples; C_j , the reference concentration for i th sample and C_{pj} represents the estimated concentration of C_{ji} (where i represents the observation).

The usual statistical parameter, indicating the quality of prediction for all the data, is the root mean square error (RMSE). RMSE values are an estimate of absolute prediction error for each component in the calibration model. However, in this work a second parameter was used to evaluate the quality of prediction: Q^2_{cum} index. The Q^2_{cum} index measures the global contribution of the n th first components to the predictive quality of the model (and of the sub-models if there are several dependent

variables). The maximum Q^2_{cum} index is equivalent to the most stable model.

A simple method used to determine the variable importance consists of calculating the VIP (variable importance for the projection) of Wold [28]. VIP summarises the contribution of each variable to the model. Descriptors with VIP superior to 0.8 were considered as relevant to explain the non-contribution of other variables.

Quality of calibration

With regard to traditional single wavelength (univariate) calibrations, figures of merit for multiwavelength calibration have been reported to quantify the quality of a given multivariate model. The method selected relies on net analyte signal calculations (NAS) [29] that is defined as the part of the measured signal that is unique for the analyte under consideration. NAS allow the estimation of the figures of merit in multivariate calibration models, such as sensitivity (SEN), selectivity (SEL), analytical sensitivity (γ) and limit of determination (LOD).

Sensitivity is defined as:

$$\text{SEN} = 1/\|b\| = \|\text{NAS}\|$$

where $\|b\|$ is the norm of the regression vector in the response vector.

Selectivity is defined as:

$$\text{SEL} = \|\text{NAS}\|/\|x\|$$

where $\|x\|$ is the spectrum containing the analyte of interest.

The analytical sensitivity γ may be defined as analogous with univariate calibration:

$$\gamma = \text{SEN}/\|\epsilon\|$$

where $\|\epsilon\|$ is the norm of the instrumental error estimated from the standard deviation of the spectral residuals.

LOD may be expressed as [30]:

$$\text{LOD} = 3 \cdot \|\epsilon\|/\text{SEN}$$

RESULTS AND DISCUSSION

Optimising conditions

The chemical structures of isosorbide dinitrate, midazolam and noradrenaline are shown in Figure 1.

Figure 2 shows the UV absorption spectra of these drugs (isosorbide dinitrate (10 $\mu\text{g}/\text{mL}$), midazolam (7.5 $\mu\text{g}/\text{mL}$) and noradrenaline (20 $\mu\text{g}/\text{mL}$), separately and in mixture, in NaCl 0.9% solution. As this figure shows, there was clear overlapping; spectral overlapping of the drugs prevents

resolution of the mixtures by direct spectrophotometric measurements. Thus, univariate analysis could not be applied to resolve them. The optimum conditions for a quantitative estimation of the compound under consideration were established via a number of preliminary experiments, as described below.

Choice of optimal spectral zone

In principle, the use of the entire spectral region is possible during multivariate calibration analysis. However, unwanted regions that do not contain information on the analytes of interest are included, and this introduces interference to the model. Spectral regions with high Q^2_{cum} indices should be selected to minimise errors because of unwanted regions in the model. Four different spectral zones were selected during the calibration process (full spectra from 200 to 320 nm and regions from 220 to 320 nm, from 200 to 300 nm and from 220 to 300 nm). The 220-300 nm spectral zone was the best model with the highest Q^2_{cum} index (see Table 1).

Influence of pH

The influence of pH values on the spectrum of each drug at a constant concentration [isosorbide dinitrate (10 $\mu\text{g/mL}$), midazolam (7.5 $\mu\text{g/mL}$) and noradrenaline (20 $\mu\text{g/mL}$) in sodium chloride 0.9% solution], was investigated separately. This study was made over a pH range of 2-10, adjusted with buffer solutions.

Although significant changes were observed in the mixture spectrum (see Figure 3), this analytical method was developed with the aim of assessing medical devices. In this context, the rest of the experiment was carried out with a pH of 4, which is the pH of the mixture obtained through clinical processes when directly used, without modifying pH of the mixture.

Selection of informative variables

In the 220-300 nm range wavelength, 119 VIP are superior to 0.8. The model was obtained by PLS regression, using only the absorbance values from these wavelengths. The results are better than with the full

Table 1: Q^2_{cum} obtained with the four wavelength ranges

Wavelength	Factors	Q^2_{cum}
200-320	14	0.9971
200-300	16	0.9971
220-320	11	0.9976
220-300	11	0.9989

Q^2_{cum} : quality of prediction index

data in the 220-300 nm range (see Table 2). The data in the 220-300 nm range wavelength were considered optimal with a similar Q^2_{cum} index (0.998) but with a better r^2 (correlation coefficient) and RMSE for each product.

In this study, it was possible to observe a large number of latent variables in the calibration models. Generally, a high number of latent variables degrade the prediction ability of the model; however, it was not the case in this study. One explanation of this apparent paradox is the complexity of the mixture, which had spectra for three drugs, without specific characteristics. For the three drugs, the absorption maxima are between 200 and 210 nm, and a plateau between 220 and 240 nm.

Multivariate methods

Resolution of ternary mixtures by applying PLS

PLS techniques are typical full-spectrum methods, more powerful than the ones based on measurement at only one wavelength, such as the direct spectrophotometric method, because the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and applicability of the quantitative spectral analysis of mixtures. The first step in simultaneously determining isosorbide dinitrate, midazolam and noradrenaline in mixture by multivariate methods involved constructing the calibration matrix. Multivariate calibration requires an experimental design of the standard composition of the calibration set that will provide the best predictions. The calibration sets contained 48 standard solutions for binary determinations in the first part, and seven standard solutions for ternary determinations in the second part, so that the concentration of each resulting drug solution was 6.67 and 33.33 $\mu\text{g/mL}$ for isosorbide dinitrate, 0.83 and 7.50 $\mu\text{g/mL}$ for midazolam and 3.33 and 23.33 $\mu\text{g/mL}$ for noradrenaline. The choices of the concentrations are defined according to clinical practice and to permit a linearity between absorbance and concentration.

Recovery study

This study was performed on prediction sets containing eight different ternary mixtures of isosorbide dinitrate,

Figure 2: UV absorption spectra of the three drugs separately and in combination

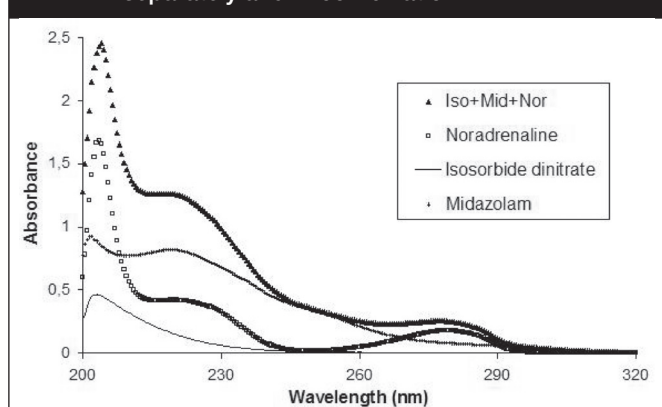
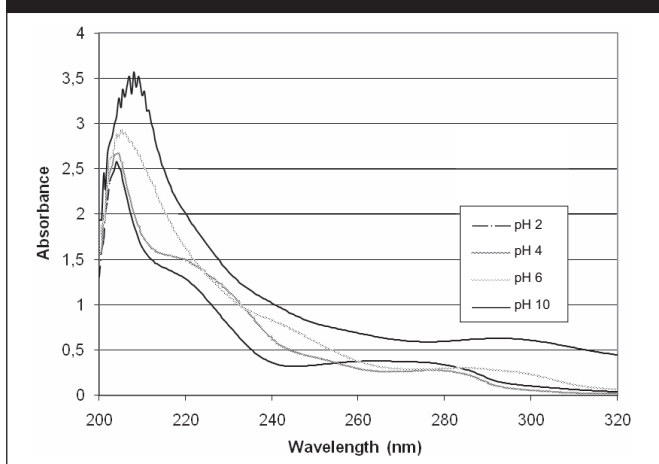


Figure 3: UV spectra of a mixture of the drugs (constant concentration) in pH range 2-10



midazolam and noradrenaline. Predicted concentrations obtained by applying the PLS method to eight ternary mixtures to determine the concentrations of isosorbide dinitrate, midazolam and noradrenaline simultaneously are given in Table 3. The prediction error of a single component in the mixture was calculated as root mean square error: the results are found in Table 4. The recovery percentage was also calculated for each component by applying PLS to ternary mixtures. The results obtained by using the constructed model for the simultaneous determination of drugs in ternary mixtures are also presented in Table 4. It can be noted that there is good agreement between experimental and predicted values for the three analytes with a recovery percentage in the 99.5-101% range.

Known concentrations of all tested samples in prediction sets were compared with concentrations predicted in constructed models, equations and r^2 , and obtained when plots of predicted versus actual concentrations were placed; actual and predicted values for all components were found to agree very well (r^2 between actual and predicted concentrations: isosorbide dinitrate: 0.9998; midazolam: 0.9996; noradrenaline: 0.9999).

Quality of calibration

The quality of calibration corresponds to the choice of concentrations for a

Table 2: R^2 obtained with all data and data with VIP superior to 0.8

Spectral zone 220-300 nm	Product	R^2	RMSE
All λ	Isosorbide dinitrate	0.9998	0.2495
	Midazolam	0.9996	0.5231
	Noradrenaline	0.9999	0.0655
Only with VIP >0.8	Isosorbide dinitrate	0.9999	0.2170
	Midazolam	0.9997	0.4566
	Noradrenaline	0.9999	0.0631

VIP: variable importance for the projection; R^2 : correlation coefficient; RMSE: root mean square error; λ : wavelength (nm)

correct sensitivity for the experiment and the definition of a reliable model.

The estimation of the figures of merit in multivariate calibration models is given in Table 5. No method allowing the simultaneous measurement of isosorbide dinitrate, midazolam and noradrenaline has been known to be published yet. On the contrary, several specific HPLC methods with UV detection have been described for isosorbide dinitrate [31] and midazolam [32-34] measurement. With these specific methods, the limits of detection are similar or smaller than in this study, around 300 versus 209 ng/mL and 10 versus 32 ng/mL for isosorbide dinitrate and midazolam respectively. Nevertheless, the limits of detection for these three drugs in this study are compatible with the outflow studies of infusion devices. For noradrenaline, HPLC was associated with electrochemical [35, 36, 37] or fluorescence detection [38]. Another great advantage in this method beside simultaneous measurement, is the speed which allows continuous measurement. This

Table 3: Results of the recovery study

Mixture	Expected Concentration			Predicted Concentration		
	Isosorbide dinitrate ($\mu\text{g/mL}$)	Midazolam ($\mu\text{g/mL}$)	Noradrenaline ($\mu\text{g/mL}$)	Isosorbide dinitrate ($\mu\text{g/mL}$)	Midazolam ($\mu\text{g/mL}$)	Noradrenaline ($\mu\text{g/mL}$)
1	23.33	5.83	23.33	22.66	4.17	23.50
2	3.33	5.83	26.66	3.11	5.83	27.00
3	20	8.33	11.66	21.26	8.54	11.53
4	23.33	3.33	10	23.49	3.29	9.81
5	26.66	5.00	6.66	26.15	4.97	6.59
6	16.66	4.16	11.66	16.96	4.13	11.76
7	30	8.33	13.33	30.14	8.46	13.23
8	16.66	3.33	26.66	17.19	3.27	27.06

Table 4: Correlation statistics of predicted versus actual values for each drug

	R ²	PRESS	Mean recovery (%)	RMSE
Isosorbide dinitrate	0.9998	2.7611	99.67 ± 1.23	0.2170
Midazolam	0.9996	2.8236	100.73 ± 4.60	0.4566
Noradrenaline	0.9999	0.3824	100.57 ± 1.54	0.0631

R²: correlation coefficient; PRESS: predicted error sum of squares; RMSE: root mean square error

Table 5: Values of sensitivity, selectivity, γ and LOD for each component

	Isosorbide dinitrate	Midazolam	Noradrenaline
Sensitivity	1.930	1.278	0.302
Selectivity	0.075	0.189	0.018
γ ($\mu\text{g/mL}$)	6.073	59.121	4.023
LOD ($\mu\text{g/mL}$)	0.494	0.051	0.746

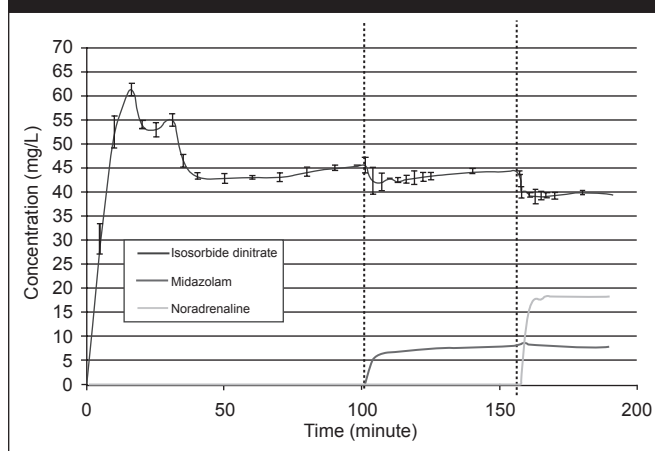
γ : analytical sensitivity (as defined in material and methods)

method seems very well adapted to the outflow evaluation in infusion devices.

Application

The first application of this method was in assessing infusion devices for anaesthesia practice. Thus, multi-infusion of the three drugs and an isotonic saline solution was simulated using a specific multi-access extension set. At the start (T_0), isosorbide dinitrate (1 mg/mL) was infused at a rate of 15 mL/hour for 15 minutes, then at 10 mL/hour for 10 minutes and then at 7 mL/hour. At $T_0 + 95$ minutes, midazolam (5 mg/mL) was started at an infusion rate of 7 mL/hour. At $T_0 + 155$ minutes, noradrenaline (2 mg/mL) was started at an infusion rate of 7 mL/hour. Isosorbide dinitrate was infused from the proximal access point, midazolam from the median one and noradrenaline from the distal access point (closest to the patient). Outgoing samples from the catheter were collected every 40 seconds for 10 minutes, then at one minute, three minutes and

Figure 4: Evolution of the three drug concentrations during multi-infusion



finally 10 minutes after the start of infusion of each product, to work out the interaction between flow rates and also to determine the physical characteristics. Samples were then analysed by applying PLS to UV spectra. The results are given in Figure 4, showing that a delay is necessary to find out the different products according to the access point used and the associated dead volume. It has been previously shown that the delay to reach the plateau concentration is directly related to the dead volume [39, 40].

CONCLUSION

According to the results of this study, the UV spectrophotometric method using PLS regression can be considered as an effective and accurate way to determine the concentrations of isosorbide dinitrate, midazolam and noradrenaline simultaneously in mixtures.

Verification of calibration produced satisfactory results showing that the predicted values were close to the expected ones (mean recovery was 99.67% for isosorbide dinitrate, 100.73% for midazolam and 100.57% for noradrenaline).

This method will enable the assessment of medical devices used for target-controlled infusion in anaesthesia.

REFERENCES

- Lauven PM, Stoeckel H, Schwilden H. A microprocessor controlled infusion scheme for midazolam to achieve constant plasma levels. *Anaesthesist*. 1982;31(1):15-20.
- White PF. Clinical uses of intravenous anesthetic and analgesic infusions. *Anesth Analg*. 1989;68(2):161-71.
- Shafer SL, Gregg KM. Algorithms to rapidly achieve and maintain stable drug concentrations at the site of drug effect with a computer-controlled infusion pump. *J Pharmacokinet Biopharm*. 1992;20(2):147-69.
- Bailey JM. A technique for approximately maintaining constant plasma levels of intravenous drugs. *Anesthesiology*. 1993;78(1):116-23.
- Miller DR. Intravenous infusion anaesthesia and delivery devices. *Can J Anaesth*. 1994;41(7):639-51.
- Gepts E. Pharmacokinetic concepts for TCI anaesthesia. *Anaesthesia*. 1998;53 (Suppl 1):4-12.
- Russel D. Intravenous anaesthesia: manual infusion schemes versus TCI systems. *Anaesthesia*. 1998;53 (Suppl 1):42-5.

8. Van Poucke GE, Bravo LJ, Shafer SL. Target controlled infusions: targeting the effect site while limiting peak plasma concentration. *IEEE Trans Biomed Eng.* 2004;51(11):1869-75.
9. Maddock J, Lewis PA, Woodward A, et al. Determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by high-performance liquid chromatography-thermal energy analysis. *J Chromatogr.* 1983;272(1):129-36.
10. Gelber L, Papas AN. Validation of high-performance liquid chromatographic methods for analysis of sustained-release preparations containing nitroglycerin, isosorbide dinitrate, or pentaerythritol tetranitrate. *J Pharm Sci.* 1983;72(2):124-6.
11. Olsen CS, Scroggins HS. High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythryl tetranitrate in various tablet forms. *J Pharm Sci.* 1984;73(9):1303-4.
12. Carlson M, Thompson RD, Snell RP. Determination of isosorbide dinitrate in pharmaceutical products by HPLC. *J Chromatogr Sci.* 1988;26(11):574-8.
13. Verma RK, Garg S. A validated high performance liquid chromatographic method for analysis of isosorbide mononitrate in bulk material and extended release formulations. *J Pharm Biomed Anal.* 2002;30(3):583-91.
14. Prue DG, Johnson RN, Kho BT. Gas-liquid chromatographic determination of isosorbide dinitrate in tablets. *J Assoc Off Anal Chem.* 1977;60(6):1341-4.
15. Turner WR, Lenkiewicz RS. Polarographic determination of isosorbide dinitrate. *J Pharm Sci.* 1976;65(1):118-21.
16. Pommier F, Gauducheau N, Pineau V, et al. Simultaneous determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by capillary gas chromatography with electron-capture detection. *J Chromatogr B Biomed.* 1996;678(2):354-9.
17. Woo D, Yen JK, Sofronas P. Quantitative analysis of 1,3,4,6-dianhydro-D-glucitol 2,5-dinitrate (isosorbide dinitrate) by infrared spectrometry. *Anal Chem.* 1973;45(12):2144-5.
18. Vasilades JV, Sahawneh T. Midazolam determination by gas chromatography, liquid chromatography and gas chromatography-mass spectrometry. *J Chromatogr.* 1982;228:195-203.
19. Rubio F, Miwa BJ, Garland WA. Determination of midazolam and two metabolites of midazolam in human plasma by gas chromatography—negative chemical-ionization mass spectrometry. *J Chromatogr.* 1982;233:157-65.
20. de Vries JX, Rudi J, Walter-Sack I, Conradi R. The determination of total and unbound midazolam in human plasma. A comparison of high performance liquid chromatography, gas chromatography and gas chromatography/mass spectrometry. *Biomed Chromatogr.* 1990;4(1):28-33.
21. Quintela O, Cruz A, Concheiro M, et al. A sensitive, rapid and specific determination of midazolam in human plasma and saliva by liquid chromatography/ electrospray mass spectrometry. *Rapid Commun Mass Spectrom.* 2004;18(24):2976-82.
22. Imai K, Tsukamoto M, Tamara Z. High-performance liquid chromatographic assay of rat-brain dopamine and norepinephrine. *J. Chromatogr.* 1977;137(2):357-62.
23. Thomas EV, Haaland DM. Comparison of multivariate calibration methods for quantitative spectral analysis. *Anal Chem.* 1990;62:1091-9.
24. Duran Meras I, Espinosa Mansilla A, Salinas Lopez F, Rodriguez Gómez MJ. Determination of triamterene and leucovorin in biological fluids by UV derivative-spectrophotometry and partial least-squares (PLS-1) calibration. *J Pharm Biomed Anal.* 2002;27:81-90.
25. Sena MM, Chaudhry ZF, Collins CH, Poppi RJ. Direct determination of diclofenac in pharmaceutical formulations containing B vitamins by using UV spectrophotometry and partial least squares regression. *J Pharm Biomed Anal.* 2004;36(4):743-9.
26. Sena MM, Poppi RJ. N-way PLS applied to simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine. *J Pharm Biomed Anal.* 2004;34(1):27-34.
27. Abbaspour A, Mirzajani R. Simultaneous determination of phenytoin, barbital and caffeine in pharmaceuticals by absorption (zero-order) UV spectra and first-order derivative spectra-multivariate calibration methods. *J Pharm Biomed Anal.* 2005;38(3):420-7.
28. Wold S. QSAR: Chemometric methods in molecular design, in PLS for multivariate linear modelling. Vol 2. van de Waterbeemd H, editor. Weinheim, Germany: Wiley-VCH; 1995. p.105-15.
29. Lorber A, Kowalski BR, Faber K. Net analyte signal calculation in multivariate calibration. *Anal Chem.* 1997;69(8):1620-6.
30. Booksh KS, Kowalski BR. Theory of analytical chemistry. *Anal Chem.* 1994;66(15):782A-91A.
31. Verma RK, Garg S. A validated high performance liquid chromatographic method for analysis of isosorbide mononitrate in bulk material and extended release formulations. *J Pharm Biomed Anal.* 2002;30(3):583-91.
32. Jurica J, Dostálek M, Konečný J, et al. HPLC determination of midazolam and its three hydroxy metabolites in perfusion medium and plasma from rats. *J Chromatogr B Anal Technol Biomed Life Sci.* 2007;852(1-2):571-7.
33. Odou P, Robert H, Luyckx M, et al. A routine HPLC method for monitoring midazolam in serum. *Biomed Chromatogr.* 1997;11(1):19-21.
34. Mastey V, Panneton AC, Donati F, Varin F. Determination of midazolam and two of its metabolites in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl.* 1994;13:305-10.
35. Brandsteterová E, Kubalec P, Krajnák K, Skacáni I. SPE-HPLC determination of catecholamines using an affinity principle. *Neoplasma.* 1996;43(2):107-12.
36. Hollenbach E, Schulz C, Lehnert H. Rapid and sensitive determination of catecholamines and the metabolite 3-methoxy-4-hydroxyphen-ethyleneglycol using HPLC following novel extraction procedures. *Life Sci.* 1998;63(9):737-50.
37. Siaghy EM, Devaux Y, Schroeder H, et al. High-performance liquid chromatographic analysis of muscular interstitial arginine and norepinephrine kinetics. A microdialysis study in rats. *J Chromatogr B Biomed Appl.* 2000;745(2):279-86.
38. Wood AT, Hall MR. Reversed-phase high-performance liquid chromatography of catecholamines and indoleamines using a simple gradient solvent system and native

- fluorescence detection. *J Chromatogr B Biomed Appl.* 2000; 744(1):221-5.
39. Lovich MA, Doles J, Peterfreund RA. The impact of carrier flow rate and infusion set dead-volume on the dynamics of intravenous drug delivery. *Anesth Analg.* 2005;100:1048-55.
40. Lovich MA, Kinnealley ME, Sims NM, Peterfreund RA. The delivery of drugs to patients by continuous intravenous infusion: modeling predicts potential dose fluctuations depending on flow rates and infusion system dead volume. *Anesth Analg.* 2006;102:1147-53.