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Rapid determination of lidocaine solutions with non-column chromatographic diode array UV spectroscopy and multivariate calibration

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Abstract

A new method for the rapid determination of pharmaceutical solutions is proposed. A conventional HPLC system with a Diode Array Detector (DAD) was used with no chromatographic column connected. As eluent, purified water (Milli Q) was used. The pump and autosampler of the HPLC system were mainly utilised as an automatic and convenient way of introducing the sample into the DAD. The method was tested on the local anaesthetic compound lidocaine. The UV spectrum (245–290 nm) from the samples analysed in the detector was used for multivariate calibration for the determination of lidocaine solutions. The content was determined with PLS regression. The effect on the predictive ability of three factors: flow, data-collection rate and rise time as well as two ways of exporting a representative UV spectrum from the DAD file collected was investigated by means of an experimental design comprising 11 experiments. For each experiment, 14 solutions containing a known content of lidocaine were analysed (0.02–0.2 mg ml^{-1}). From these 14 samples two calibration sets and two test sets were made and as the response in the experimental design the Root Mean Square Error of Prediction (RMSEP) values from the predictions of the two test sets were used. When the factor setting giving the lowest RMSEP was found, this setting was used when analysing a new calibration set of 12 lidocaine samples (0.1–0.2 mg ml^{-1}). This calibration model was validated by two external test sets, A and B, analysed on separate occasions for the evaluation of repeatability (test set A) and determination over time (test set B). For comparison, the reference method, liquid chromatography, was also used for analysis of the ten samples in test set B. This comparison of the two methods was done twice on different occasions. The results show that in respect of accuracy, precision and repeatability the new method is comparable to the reference method. The main advantages compared with liquid chromatography are the much shorter time of analysis (< 30 s) as well as the automatic and simple analytical procedure and the low consumption of organic solvents.

Keywords: Non-column; Diode array UV spectroscopy; Multivariate calibration; Lidocaine; PLS

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1. Introduction

In analytical chemistry it is always of interest to establish analytical methods capable of analysing precisely and accurately a large number of samples in a short time period. Spectroscopic techniques generate large amounts of data with a short period of analysis. By means of chemometrics tools the amount and quality of the information in spectra can be increased.

If spectrometric techniques are combined with automation, it is possible to develop fast and automatic analytical methods. Spectrometric techniques can be used together with multivariate calibration to determine various compounds. For the determination of liquid samples, ultraviolet-visible spectroscopy can be used [1–9]. Recently a new method was developed for the determination of content and identity of lidocaine solutions with UV–Vis spectroscopy and multivariate calibration [10].

This method was developed on a stand-alone spectrophotometer equipped with open-topped, regular fused quartz cells that were cleaned and filled manually. It is, however, desirable to have a more automatic approach for this type of analysis. One way of achieving this would be to use a spectrophotometer with a flow-through cell. Another approach would be to use a flow injection analysis system (FIA) coupled with a diode array detector (DAD). The latter approach has been used for rapid determination of metal ions in water [11–13].

In this paper we have chosen another convenient way of obtaining a highly automated UV–Vis spectroscopic system for multivariate calibration, a modern conventional HPLC system with a DAD detector. Modern HPLC systems have highly automated autosamplers that together with the pump can be used for introducing the sample into the spectrophotometer, the DAD. With no chromatographic column connected, the sample can be rapidly analysed. In this way one obtains a highly automated and fully computer-controlled UV–Vis spectrophotometer capable of analysing large number of samples in a relative short time period. Hence, the purpose of the present work is to propose an alternative approach for the use of a conventional DAD together with the other parts of the HPLC system for multivariate calibration and determination of pharmaceutical solutions without any separation. Since multivariate calibration using many variables generally is more robust to instrumental variation as well as interference from of other UV absorbing compounds it was chosen instead of univariate calibration in this study. The method was tested on the local anaesthetic agent lidocaine.

The predictions results obtained were compared with the reference method, HPLC.

The new method is referred to as the DAD method in the following text.

2. Experimental

2.1. Instrumentation

The HPLC system used was a Dionex Summit HPLC system together with the chromatographic data system (CDS) CHROMELEON ver 6.11 (Dionex Corporation). The DAD was a Dionex PDA 100 DAD. This is a DAD with a wavelength range of 190–800 nm and the detector is equipped with 1024 diodes. The bandwidth used throughout this study was 1 nm. The autosampler was a Dionex ASI 100T, an autosampler of carousel type with a variable injection volume. The injection volume used in this study was 20 μl. The pump was a Dionex P580HPG, a binary high-pressure gradient pump of two-plunger type with a dynamic mixer. When the HPLC system was used without any chromatographic column, a back-pressure tube (id 0.1 mm, length 6 m) was used to obtain pressure for the pump to work against. This tube was connected between the autosampler and the DAD. As mobile phase, purified water (Milli-Q) was used. The instrument set-up is described schematically in Fig. 1.

The spectral window used was the same as the one used in the previous investigation [10], 245–290 nm. In this wavelength region lidocaine shows a characteristic shape of the absorbance spectrum (Fig. 2). HPLC experiments were carried out using the same instrument set-up but with a chromatographic column connected. The column was a

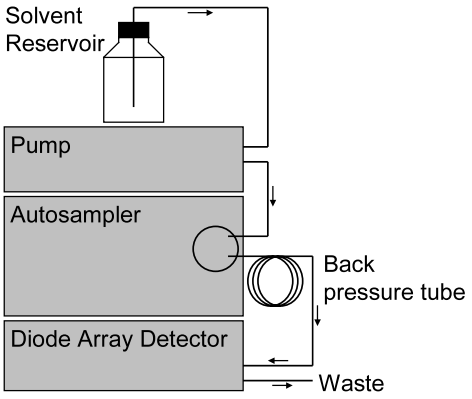

Fig. 1. Instrumental set-up.

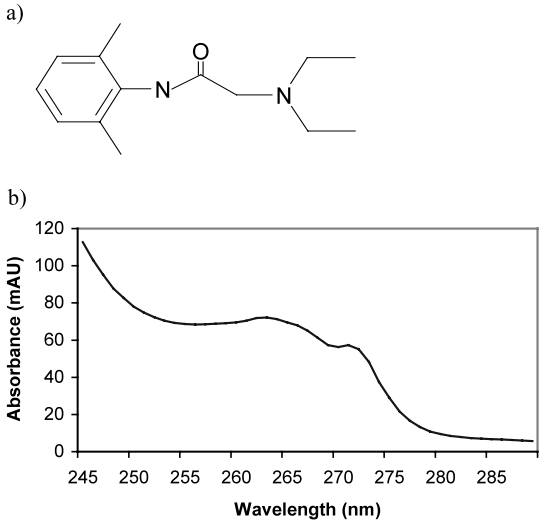

Fig. 2. Structure formula (a) and UV spectrum (b) of lidocaine (0.15 mg ml^{-1}).

μBondapack C_{18} (150 × 3.9 mm), the flow was 1.4 ml min^{-1} and the detector wavelength was 240 nm. The mobile phase consisted of 60% v/v acetonitrile (AcN) and 40% v/v phosphate buffer pH 8.0. The eluent was degassed with helium before use. The determination of lidocaine was carried out by means of linear regression with an external standard. For the weighing of substances for the sample preparation, a calibrated balance, Sartorius MC-1, was used. For the collection of DAD data and for the HPLC experiments, CHROMELEON was used. For creation of the experimental design and its evaluation with MLR, Umetrics software MODDE 5.0 was used. For the multivariate calibration Umetrics software, SIMCA-P 8.1 was used. As the interface for data transfer between CHROMELEON and SIMCA, MS EXCEL was used.

2.2. Reagents

The chemicals used in this study were either of analytical grade or of spectroscopic quality. Phosphate buffer pH 8.0 was prepared by mixing 1.3 ml of 1 M sodium dihydrogen phosphate and 32.5 ml of 0.5 M disodium hydrogen phosphate with water (Milli Q, Waters). Analytical reagent grade AcN was used. Lidocaine hydrochloride was supplied by AstraZeneca Bulk production, Södertälje. HPLC grade water provided by a Millipore Milli-Q water filtration/purification system was used. Lidocaine was weighed and dissolved in Milli-Q water, after which the solutions were further diluted with the same solvent with a digital dilutor, Hamilton Microlab 1000, to the concentration range 0.02–0.25 mg ml^{-1}. The sample solutions were made in this way at different concentrations.

2.3. Data sampling

The DAD starts to scan UV spectra immediately after the sample has been injected and the analysis is started. The shape of the DAD file collected for a sample is described in Fig. 3. This figure shows an isoplot from CHROMELEON of a lidocaine sample where wavelength, time and absorbance are plotted for a sample containing 0.15 mg ml^{-1} of lidocaine. The lidocaine has reached the detector after about 0.2 min (flow 1.5 ml min^{-1}) and the UV spectrum in the chosen interval 245–290 nm has been collected. The level of the absorbance is shown with different colours, where white, purple and red show the regions of highest absorbance.

Before the sample has reached the detector, the UV spectrum collected contains only noise since it is the mobile phase (Milli-Q water) that is scanned (Fig. 3b). When the sample plug reaches the detector cell, the UV spectrum of the lidocaine

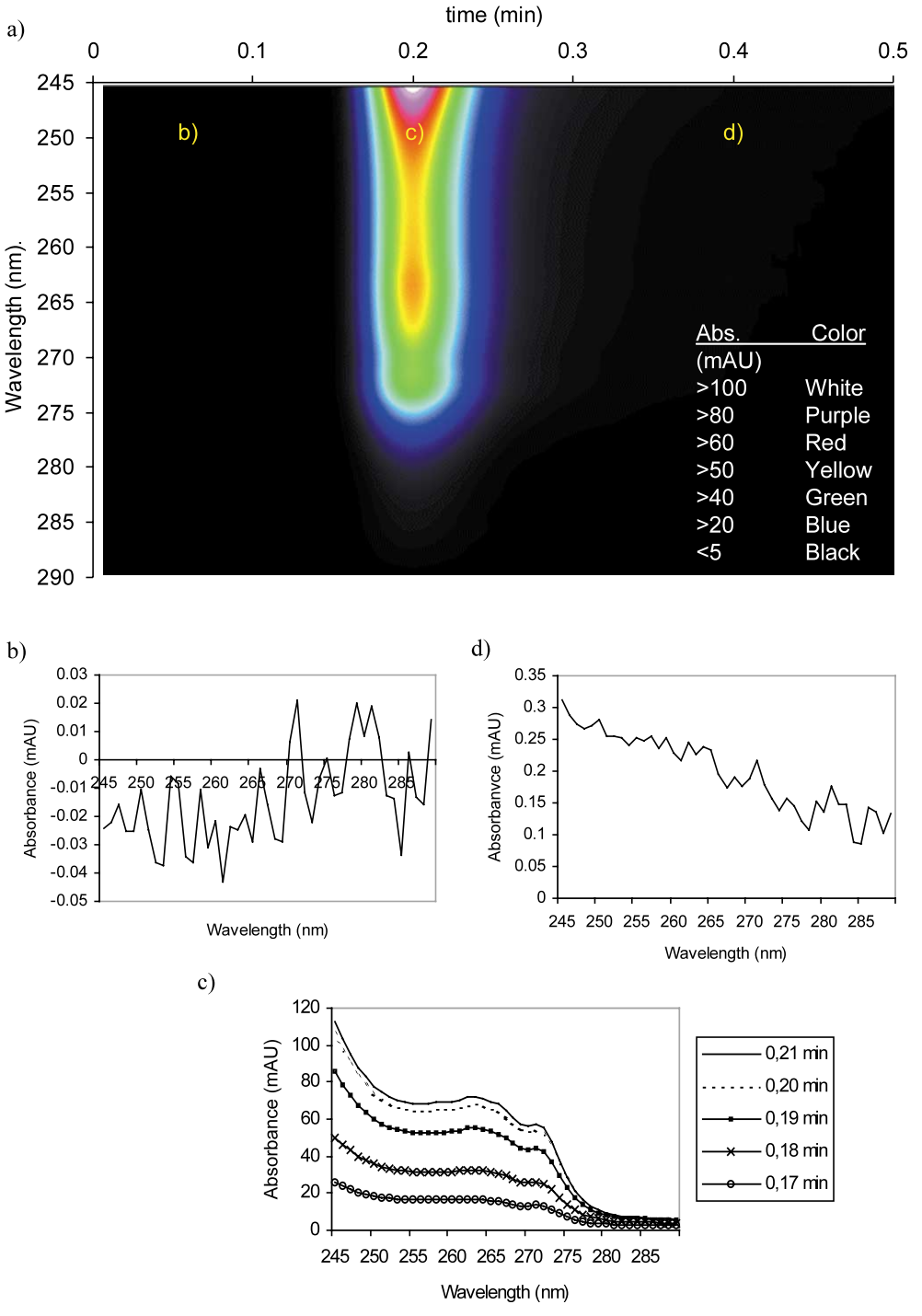

Fig. 3. Schematic description of the shape of the UV spectra collected at different time of the analysis (0.15 mg ml^{-1} lidocaine). a) isoplot of the collected DAD file taken from CHROMELEON, b) UV spectrum at 0.05 min, c) UV spectrum around SMIT and d) UV spectrum at 0.4 min.

sample appears and increases in intensity when the lidocaine concentration increases. When the centre of the sample plug is in the detector cell, the UV spectrum reaches its maximum intensity (Fig. 3c). In this paper this is called the spectral maximum intensity time (SMIT) and is equivalent to the retention time for a peak in liquid chromatography. When the centre of the sample plug has passed through the detector, the intensity of the UV spectrum of lidocaine decreases and is replaced with noise when the last portions of sample has been flushed out of the detector cell (Fig. 3d).

The resulting data matrix from the collected DAD-data contains several UV spectra from the wavelength region chosen. The number of UV spectra collected depends on the data-collection rate as well as the run time of each analysis. With a data-collection rate of 10 Hz and a run time of 30 s, 300 UV spectra are collected for each DAD file. The spectral window used in this study was 245–290 nm and the resolution 1 nm. This means that each UV spectrum contained 45 variables and, depending on the data-collection rate and the run time, each DAD file contained between 45×60 to 45×600 data points.

In this work two ways of exporting a representative UV spectrum from the DAD files collected with CHROMELEON was tested. The two procedures and the DAD data matrix are schematically described in Fig. 4. The first way of exporting a representative UV spectrum was to find in each DAD file the spectrum that had the highest intensity, i.e. the UV spectrum at SMIT, and export this. The other way of exporting a representative UV spectrum was to calculate the average spectrum of the whole DAD file. These two approaches were performed with the help of macros written in MS EXCEL. The UV spectra were then imported into SIMCA-P, where the calibration model and predictions were made.

The time for the sample to reach the detector is controlled by the flow rate. Higher flow rates also lower the SMIT. This is also dependent on the length and inner diameter of the back-pressure tube. Three different flow rates were tested: 0.5, 1 and 1.5 ml min^{-1}. With the back-pressure tube, used the SMITs obtained at the different flows were around 36 s at 0.5 ml min^{-1}, 20 s at 1 ml min^{-1} and 14 s at 1.5 ml min^{-1}. The SMIT was not completely stable, a small variation around ± 1 s being observed. Some widening of the sample plug was also observed. The higher the flow, the less the widening of the sample plug.

3. Results

3.1. Experimental design

An experimental design was used to investigate how three factors as well as the two ways of exporting a representative UV spectrum influenced the predictive ability. It was of interest to develop a fast analytical method, and since the time of analysis in the method proposed is dependent on the flow rate this was one of the factors examined. Two parameters that have to be decided when using CHROMELEON for collection of DAD files are the data-collection rate and rise time. Rise time is a measure of how quickly the detector responds to a change in signal and in chromatography is defined as the time it takes for the output signal to rise from 10% of its final value to 90% of its final value [14].

Hence the three factors investigated were the flow rate (ml min^{-1}), the data collection rate (Hz) and the rise time (s). All three factors were defined and controlled in CHROMELEON. A full factorial design at two levels was used, resulting in 11 experiments, eight plus three replicates in the centre of the factor interval. The factor settings were flow rate 0.5–1.5 ml min^{-1}, data collection rate 2–10 Hz and rise time 1–5 s. The experimental design is shown in Table 1.

In each of the 11 experiments 14 samples of a known amount of lidocaine (0.02–0.2 mg ml^{-1}) [10] were analysed with the DAD method. For each sample, the UV spectrum between 245 and 290 nm with a resolution of 1 nm was collected (45 X variables). Pre-runs with the three flow rates indicated an appropriate run-time of 1 min. With a run-time of 60 s, depending on the data-collection rate used, the DAD file contained between 120 (2 Hz) and 600 (10 Hz) UV spectra.

The concentration of the samples used is shown in Table 2. With these samples two separate sets of

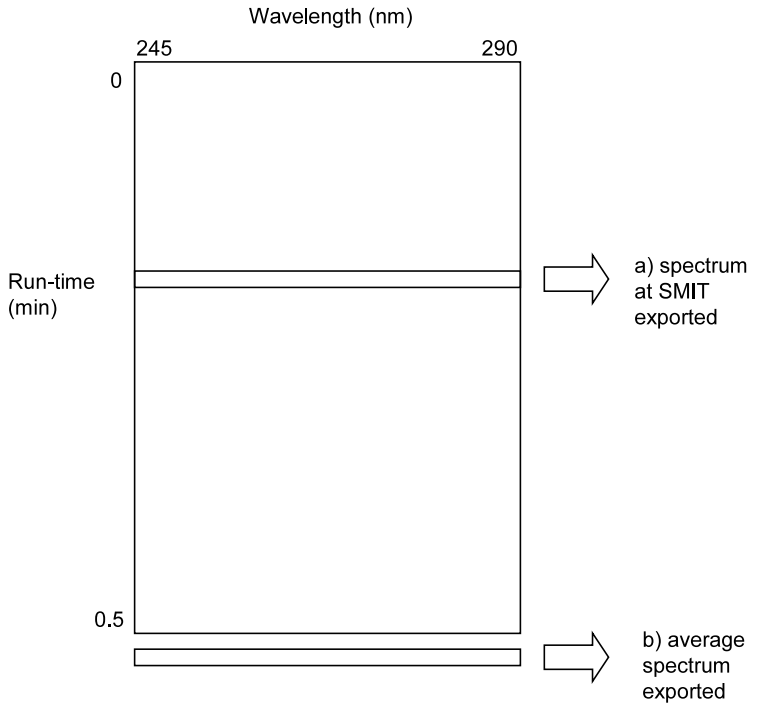

Fig. 4. Schematic picture of the two approaches used to export the UV spectrum from the DAD file. a) export of the spectrum at the spectra maximum intensity time (SMIT) b) export of the calculated average spectrum of the DAD file.

calibrations and predictions were made for each experiment in the experimental design. In these calibrations the concentration of lidocaine was used as the Y variable. First, samples 1–7 were used as calibration samples and a PLS model was made and used to predict the lidocaine content in the other seven samples (nos. 8–14) analysed in that experiment. Then the reversed approach was used, samples nos. 8–14 being used for calibration and prediction of samples nos. 1–7 in that experiment. From the DAD files of the 14 samples in the 11 experiments, the representative UV spectra for each sample were exported with the two methods mentioned above—spectrum at SMIT and average

Table 1
Experimental design used for investigation of the effect of instrumental parameters on predictive ability

Experimental number	Flow (ml min^{-1})	Data collection rate (Hz)	Rise time (s)
1	0.5	2	1
2	1.5	2	1
3	0.5	10	1
4	1.5	10	1
5	0.5	2	5
6	1.5	2	5
7	0.5	10	5
8	1.5	10	5
9	1	6	3
10	1	6	3
11	1	6	3

Table 2
Concentration of samples 1–14 used for calibration and prediction in the experimental design

Sample number	Concentration (mg ml^{-1})
1	0.0208
2	0.0507
3	0.0812
4	0.1
5	0.12
6	0.161
7	0.2
8	0.0256
9	0.0514
10	0.0962
11	0.129
12	0.160
13	0.177
14	0.201

spectra. In this way two calibration models and two test sets were used for each of the two file export methods in each of the 11 experiments. All UV spectra were mean centred before modelling, and no other pre-treatment was used in the experimental design study. All PLS models constructed contained one PLS component which explained $>99\%$ of the variation in the X data.

As responses for the 11 experiments, the Root Mean Square Error of Prediction (RMSEP) [15] from the predictions of the samples in the test sets was used:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_{\mathrm{pred}} - y_{\mathrm{obs}})^2}{n}} \quad (1)$$

where y_{pred} is the predicted concentration in the sample, y_{obs} is the observed or reference value of the concentration in the sample and n is the number of samples in the test set.

Predictions of samples 8–14 with a calibration model from samples 1–7 gave RMSEP_1 and predictions of samples 1–7 with a calibration model from samples 8–14 gave RMSEP_2. Hence four sets of 11 RMSEP values were obtained as responses in the experimental design: RMSEP_1 and RMSEP_2 for spectra exported at SMIT and RMSEP_1 and RMSEP_2 for the average spectra exported. The range of the RMSEP values found is described in Table 3. From these results it can be seen that the two ways of exporting a representative UV spectrum gave similar results. The experimental design was evaluated with multiple linear regression (MLR) with a linear model. Before evaluation the variables were scaled to unit variance. The effect is described below by using a mathematical model that expresses the response as a function of the level of each factor. The normalised regression coefficients found were for the spectra at SMIT:

$$\mathrm{RMSEP}_1 = 3.6\times10^{-3} - 3.3\times10^{-4}F - 1.7\times10^{-5}D + 5.9\times10^{-4}R \quad (2)$$

$$\mathrm{RMSEP}_2 = 3.5\times10^{-3} - 5.3\times10^{-4}F - 3.2\times10^{-4}D + 1.3\times10^{-3}R \quad (3)$$

and for the average spectra:

$$\mathrm{RMSEP}_1 = 4\times10^{-3} - 4.6\times10^{-4}F - 3.4\times10^{-5}D + 8.4\times10^{-4}R \quad (4)$$

$$\mathrm{RMSEP}_2 = 4.4\times10^{-3} - 4.1\times10^{-4}F - 8.4\times10^{-4}D + 1.7\times10^{-3}R \quad (5)$$

where F is the flow rate, D the data-collection rate and R the rise time. The rise time was the factor that had the highest effect on the prediction ability and it should be set as low as possible to obtain a high predictive ability. The data-collection rate and flow rate had insignificant regression coefficients and had no influence on the prediction ability. The data-collection rate affects mainly the size of the data file. Higher data-collection rates give larger data files since spectra are taken at shorter time intervals. The flow rate affects the time of analysis since it controls the time for the sample to reach the detector. From the results

Table 3
Overview of the ranges of RMSEP values obtained for the eleven experiments in the experimental design with the two ways of exporting a representative UV spectrum.

	UV spectrum at SMIT	Average UV spectrum
RMSEP_1	0.002–0.005	0.002–0.006
RMSEP_2	0.001–0.006	0.001–0.007

obtained it was seen that a flow of 1.5 ml min^{-1} gave as good predictions as a lower flow.

To investigate whether a rise time of less than 1 s gave a better predictive ability, two new experiments with the 14 samples were performed with the same setting for flow and data collection rate. The factor setting for these two experiments was flow rate 1.5 ml min^{-1}, data-collection rate 10 and rise times 0.5 and 0.1 s, respectively. The result showed, however, that the predictions did not improve ($RMSEP_1$ and $RMSEP_2$: 0.004–0.01 for SMIT spectra and 0.005–0.009 for average spectra).

Hence the two file-export methods gave similar results; however, since the average spectrum was easier to handle with the EXCEL macros, the final choice of method for exporting a representative UV spectrum from the DAD file was to use the average spectra.

The final factor setting chosen was flow rate 1.5 ml min^{-1}, data-collection rate 10 Hz and rise time 1 s and export of the average spectra. With a flow rate of 1.5 ml min^{-1} the SMIT is about 0.2 min and a run-time of 0.5 min is appropriate. No further investigation concerning the factor setting was made in this study.

3.2. Calibration model

When the final factor setting and the best way of exporting a representative UV spectrum from the DAD file had been chosen, a new calibration model was constructed with PLS regression on a calibration set of 12 spectra from samples of a known content of lidocaine. The calibration model consisted of 45 (245–290 nm) X variables and 12 samples. In the reference method for determination of lidocaine, HPLC, the recommended concentration range is 0.1–0.6 mg ml^{-1}. Since a comparison between HPLC and the DAD method was intended the concentration range in this part of the study was 0.1–0.2 mg ml^{-1}. The samples in the calibration set (Table 4) were analysed once with the factor setting mentioned above and from the collected DAD files the average spectra were exported.

Table 4
Second calibration set (samples 1–12) and HPLC standard solutions (S1–S5).

Calibration sample number	Concentration (mg ml^{-1})
1	0.102
2	0.113
3	0.122
4	0.127
5	0.142
6	0.152
7	0.160
8	0.170
9	0.179
10	0.189
11	0.191
12	0.205
S1	0.102
S2	0.128
S3	0.152
S4	0.180
S5	0.205

3.3. Validation

The calibration model was validated with two external test sets, A and B, which were analysed on separate occasions in a period of 1–2 months after the calibration model had been made. Test set A was analysed once and test set B twice. The reason for analysing the test sets after the calibration set was to see whether the calibration model could be used for later analysis of samples. This is a major issue when using multivariate calibration, to construct a calibration model on one occasion and validating it with external test sets analysed on different occasions. This should be done in order to see that the calibration works over time, when instrumental parameters can vary.

When using UV–Vis spectroscopy for multivariate calibration and determination, the energy of the lamps in the spectrophotometer can affect the predictions [10]. This is more commonly seen when the calibration and test-set analysis is performed on different occasions because of the changes in instrumental parameters at the different times of analysis. In this study orthogonal signal correction (OSC) [16] was used for the calibration set and external test sets A and B in order to minimise the effect of the instrumental bias on

predictive ability. The predictions obtained were better with OSC than without the spectral pretreatment (results not shown here).

External test set A contained three samples with a known content of lidocaine (0.1, 0.15 and 0.19 mg ml^{-1}) that were analysed ten times each in order to obtain an estimate of repeatability.

External test set B consisted of ten samples with a known content of lidocaine (0.1–0.19 mg ml^{-1}) that were analysed twice on separate occasions with the DAD method as well as with HPLC for comparison. Hence the ten test-set samples in test set B were analysed with the two methods on two separate occasions to obtain a comparison over time of the two methods.

For the determination with HPLC, five external standard solutions of 0.1–0.2 mg ml^{-1} were used (Table 4). These solutions were diluted from the stock solutions for each analysis. The HPLC analysis of the content of lidocaine in the ten test-set samples was performed with linear regression, with samples S1–S5 as external standards.

For the DAD method, the average UV spectra were exported in the same way as for the calibration set and imported into SIMCA-P, where OSC filtration and the prediction of the content with the calibration model were carried out.

4. Discussion

The experimental design study showed that predictions of the lidocaine content with the DAD method was possible, giving RMSEP values of 0.001 as the best predictions for a test set in the range (0.02–0.2 mg ml^{-1}). This study gave the factor setting and the method of exporting the representative UV spectra of each DAD file, and these settings were used in the rest of this work.

In Tables 5 and 6 the prediction results from external test sets A and B can be found. The accepted reference value is the calculated concentration from the weighing and dilution of these samples. In Table 5 the repeatability of the DAD method was tested by analysing three samples with different concentrations of lidocaine ten times. This analysis was performed approximately 1 month after the calibration model was analysed. The result showed that the repeatability for the determination had a relative standard deviation (RSD) below 1.5%. In Table 6 the predicted result for the determination of the ten test set samples in external test set B on two occasions with the two methods is shown. The first analysis of external test set B was done around 5 weeks after the calibration model was created, and the other analysis around 8 weeks after the construction of the calibration model. The results show that the reference method HPLC has slightly better predictions, although the overall results of the two methods are comparable. The RMSEPs for the prediction of the test-set samples with HPLC were 0.0009 and 0.0034. For the DAD method, they were 0.0034 and 0.0042.

The RMSEP values obtained for the DAD method are slightly higher than the lowest RMSEP value found in the experimental design study (0.001). One possible explanation for this is that the samples in test set B were not analysed on the same occasion as the calibration set. In the experimental design study the calibration samples and the test-set samples were analysed on the same occasion, involving a smaller effect of fluctuating lamp energy and other instrumental parameters.

There are a number of advantages of the DAD method proposed. The most obvious one is the short time of the analysis. In the second part of this study a run-time of 30 s for each sample at a flow rate of 1.5 ml min^{-1} was used, although the sample was actually analysed after about 12–14 s, when it had reached the detector cell. Hence it would have been possible to decrease the run-time to about 15–20 s without interfering with the sample plug. The width of the sample plug was around 4 s at a flow rate of 1.5 ml min^{-1}. The total time of analysis per sample in this study was 30 s for the actual analysis and about 30 s for the autosampler between each injection. This makes a total time of analysis of around 1 min per sample, representing an analytical capacity of about 60 samples per h. The run-time of each sample can be speeded up with either a higher flow rate, a shorter or wider back-pressure tube or by placing the back-pressure tube after the detector. However, since the injection procedure of the autosampler takes around 30 s, no large time-saving can be

Table 5
Predictions obtained for test set A

Sample number	Accepted reference value (mg ml^{-1})	Predicted concentration (mg ml^{-1})	Mean of predicted concentration (mg ml^{-1})	RSD (concentration) (%)
1(a)	0.11	0.113	0.112	1.28
(b)		0.114		
(c)		0.113		
(d)		0.112		
(e)		0.111		
(f)		0.110		
(g)		0.110		
(h)		0.112		
(i)		0.111		
(j)		0.110		
2(a)	0.13	0.126	0.127	0.94
(b)		0.126		
(c)		0.127		
(d)		0.126		
(e)		0.127		
(f)		0.127		
(g)		0.128		
(h)		0.129		
(i)		0.129		
(j)		0.126		
3(a)	0.18	0.179	0.181	0.74
(b)		0.179		
(c)		0.180		
(d)		0.181		
(e)		0.181		
(f)		0.182		
(g)		0.182		
(h)		0.183		
(i)		0.182		
(j)		0.181		

achieved by reducing the run-time more than this. Rather a completely automatic data transfer procedure of the representative UV spectrum of each sample would save more time through a shorter total time of analysis. Although the MS EXCEL macros used in this study were fast, a better approach would be to have the multivariate calibration model as close as possible to the CDS, i.e. in conjunction with the CDS. One way of achieving this would be to be able to export the calibration model into CHROMELEON and make the predictions there and hence use the PLS model directly in the CDS.

The other great advantage is that the automation has already been done since an HPLC autosampler is used. In this way no work at all had to be done on the automation of the method with respect to the analysis.

A third advantage of the DAD method proposed is the lack of organic solvents needed. Since the eluent is only used as a carrier of the sample into the detector, no mobile phase with organic solvents is needed, i.e. no harmful solvents. In this study Milli-Q water was used. Also the practical part of the analysis is simplified compared with ordinary HPLC since no complex mobile phase or any external standard solutions are needed.

One benefit arising from the multivariate evaluation of the DAD spectra is that it is possible to detect and obtain diagnostics of outliers. It is possible, for instance, to use Soft Independent Modelling of Class Analogy (SIMCA) [17,18] in

Table 6
Predictions obtained for test set B.

Test set sample (number)	Accepted reference value (mg ml^{-1})	HPLC predicted concentration (mg ml^{-1})	DAD method predicted concentration (mg ml^{-1})
1	0.107	0.109	0.108
2	0.114	0.108	0.110
3	0.123	0.121	0.117
4	0.131	0.132	0.127
5	0.147	0.144	0.147
6	0.151	0.150	0.144
7	0.159	0.162	0.155
8	0.164	0.164	0.162
9	0.180	0.185	0.181
10	0.190	0.193	0.192
		RMSEP:0.0033	RMSEP:0.0042
1	0.107	0.106	0.105
2	0.114	0.114	0.111
3	0.123	0.122	0.120
4	0.131	0.131	0.129
5	0.147	0.146	0.145
6	0.151	0.151	0.144
7	0.159	0.161	0.156
8	0.164	0.164	0.162
9	0.180	0.182	0.182
10	0.190	0.192	0.188
		RMSEP:0.0009	RMSEP:0.0034

conjunction with the calibration model to diagnose how well new samples fit within the calibration model. This has been shown in a previous work [10].

Another factor that may affect the predictive ability is the injection volume. In this work an injection volume of 20 μl was used throughout the study and no investigation was made of the effect of a different injection volume on the predictive ability. It is also possible that there are other ways of exporting a representative UV spectrum from the DAD file. For instance, a calculation of the average spectra over the sample plug instead of the over the whole DAD file could be an alternative. Preliminary results showed that similar predictions might be achieved (results not shown here).

In this study there was no cleaning of the detector cell between the scanning of the samples. The time taken between the injections by the autosampler was sufficient for rinsing the detector cell of the previous sample. At the beginning of each sample the detector was auto-zeroed, no other zeroing of the detector being used.

With further development of this analytical approach the analytical capacity can be increased, which may lead to a more efficient method. A suitable term for this type of analytical method could be High Capacity Analysis (HCA). Our approach for an HCA method is as follows:

- spectroscopic analysis;
- chemometric data evaluation;
- high level of automation.

The time of analysis for an HCA method would typically be in the range < 0.5 min sample, meaning an analytical capacity of > 120 samples per h.

The high speed of this type of analytical method makes it attractive for screening purposes or for the routine determination when the number of samples is large.

Since HPLC is a very common analytical technique used world-wide, there should be ample

scope for using this type of analytical method with DAD detection.

5. Conclusion

This work shows that multivariate calibration for rapid determination of pharmaceutical solutions can be done with non-column chromatographic diode array detection.

The results obtained show that the proposed method, in terms of accuracy, precision and repeatability, is comparable with the reference method HPLC. In respect of analytical time as well as simple analytical procedure, the proposed method is much faster. The main advantage of the proposed DAD method is the high sample throughput that can be further developed into a HCA method.

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