FULL PAPER
THE ANALYST
www.rsc.org/analyst

Determination of the content and identity of lidocaine solutions with UV–visible spectroscopy and multivariate calibration

Kent Wiberg,*[ab] Anders Hagman,[a] Peter Burén[c] and Sven P. Jacobsson[ab]

[a] *AstraZeneca R&D Södertälje, Södertälje, SE-151 85 Sweden*
[b] *University of Stockholm, Department of Analytical Chemistry, Stockholm, SE-106 91 Sweden*
[c] *AstraZeneca Tablet Production, Södertälje, SE-151 85 Sweden*

Received 19th March 2001, Accepted 18th May 2001
First published as an Advance Article on the web 21st June 2001

A method is proposed for the determination of the content and identity of the active compound in pharmaceutical solutions by means of ultraviolet-visible (UV–Vis) spectroscopy, orthogonal signal correction (OSC) and multivariate calibration with soft independent modelling of class analogy (SIMCA) classification and partial least squares (PLS) regression. The content was determined with PLS regression and the identity with PLS regression and SIMCA classification. The method was tested on the local anaesthetic compound lidocaine. For the validation, external test sets of both manufactured sample solutions and samples from a stability study were used. For comparison with this new method, liquid chromatography was used as a reference method. The results show that in respect of accuracy, precision and repeatability, the new method is comparable to the reference method. The main advantage over liquid chromatography is the much shorter time of analysis and the simpler analytical procedure. An estimate of the analysis time saved with the proposed method compared with using liquid chromatography, together with practical considerations, is given.

Introduction

In the pharmaceutical industry the determination of content and identity of the active compound in pharmaceutical solutions is a very common type of analysis. This analysis is often made by means of high-performance liquid chromatography (HPLC). The determination of content is made using standard solutions and linear regression. Comparison of the retention times between standard and sample is used to confirm identity.

However, this approach has some disadvantages. One drawback is the determination of the identity by means of the chromatographic retention time, which in some cases is not sufficiently reliable as far as identification is concerned. It is possible, for instance, for a degradation product or an impurity to have the same chromatographic retention time as the active compound. The other disadvantage of the chromatographic approach is the time associated with each analysis. Even though fast liquid chromatography is becoming more common, many used methods in the pharmaceutical industry employ more conventional and slower HPLC methods.

A faster way of performing the determination of content and identity is by using spectroscopic techniques together with the use of chemometrics. This approach is frequently described in the literature, and common spectroscopic techniques used for multivariate determination of content are infrared (IR) spectroscopy in the range 4000–400 cm^{-1} and near-infrared (NIR) spectroscopy in the range 780–2500 nm. In recent years, the use of IR and NIR spectroscopy has become well established and standardised guidelines have been published.[1]

Applications of this type using UV–Vis spectroscopy are, however, not as common in the literature as applications using NIR and IR. However, with the use of chemometric tools such as partial least squares (PLS) regression it is possible to obtain sufficient information from a UV–Vis spectrum to be able to determine the contents of various compounds.[2–8]

The purpose of this paper is to propose an alternative approach for the determination of content and identity of pharmaceutical solutions by means of UV–Vis spectroscopy and multivariate calibration. The method was tested on the local anaesthetic drug lidocaine. The prediction results obtained were compared with the reference method HPLC. The new method is referred to as the UV method in the following text.

Theory

Multivariate calibration

The term multivariate calibration refers to the process of constructing a mathematical model that relates a property such as content or identity to the absorbances of a set of known reference samples at more than one wavelength. A calibration model, *i.e.*, a mathematical expression, is determined from a set of samples of known content and identity, the calibration set. This can be done by means of PLS, and the resulting model is used to predict the content and identity of new unknown samples from their digitised UV–Vis spectra. PLS is a regression technique that has been used in many different fields of chemistry and the theories behind it have been thoroughly described elsewhere,[9,10] together with the theories of multivariate calibration.[11,12]

The best calibration is obtained when the calibration model contains all the sources of variation that can occur in the actual measurement.

SIMCA classification

Soft independent modelling of class analogy (SIMCA) is a technique that uses principal component analysis (PCA) or PLS for classification.[13–16] Each class is modelled by a separate PCA analysis. From the residuals of the samples in the calibration set, a confidence region for the class is constructed around the principal component. New objects are regarded as a member of the class if their distance from the principal component space does not exceed a critical limit defined by the confidence region.

 DOI: 10.1039/b102545g

Based on the residual variance of the objects in the calibration set, the residual standard deviation (s_0) is calculated (pooled residual standard deviation):

$$s_0 = \sqrt{\frac{\sum_{k=1}^{m}\sum_{i=1}^{p} e_{ik}^{\,2}}{(m-1-r)(m-r-1)}} \quad (1)$$

where e_{ik}^2 is the residual of object k in the calibration set at variable i, m is the number of observations in the calibration set, p is the number of variables and r is the number of principal components. The number of degrees of freedom given in eqn. (1) is used when the number of observations is less than the number of variables, $m \leq p$.[14,15]

The absolute residual standard deviation (s_i) for an object in the calibration set when $m \leq p$ is given by

$$s_i = \sqrt{\frac{\sum_{i=1}^{p} e_{ik}^{\,2}}{(m-1-r)}} \quad (2)$$

The ratio s_i/s_0 in the software used in this study, Simca P 7.01, is called the normalised distance to model in X space and in the following text is denoted DmodX.

The normalised distance to model in X space values for new objects are calculated from the ratio s_i/s_0 and is called DmodXPS in the software. However, if the predicted score value of a new object is outside the model score range, the DmodXPS value is calculated as a combined measure of the object's residual standard deviation (s_i) and the distance of its score from the normal score range of the model.[17–19]

In this study, no critical limit was used for classification; instead, simple comparisons between the calculated distance to model values for the samples in the external test sets compared with the calibration set were used. Higher values of DmodXPS of the samples in the test sets compared with the calibration set give a negative identity or outlier.

The distance to model values calculated from the PLS regression was in this way used for the SIMCA classification.

One advantage of using this type of outlier detection is that the DmodXPS value gives a measurement of how the spectra of the sample fit into the multivariate calibration model. This is a major advantage of the multivariate approach over univariate calibration.

Data pre-treatment

Several types of pre-treatments of spectroscopic data in multivariate calibration are described in the literature. In this study, the following pre-treatments were tested: multiplicative scatter correction (MSC),[12] standard normal variate (SNV)[20] and orthogonal signal correction (OSC).[21] OSC removes variations from X that do not contain any information about Y, *i.e.*, removing data in X that is orthogonal against Y. Because of this OSC also give a simpler calibration model with fewer components.

Experimental

Instrumentation

The spectrophotometer used was a Unicam UV4 together with the software Vision 32 version 1.04. This is a double-beam spectrophotometer with a wavelength range of 190–600 nm and a minimum bandwidth of 0.2 nm, equipped with a tungsten lamp (325–600 nm) and a deuterium lamp (190–325 nm). Open rectangular fused quartz cells with a pathlength of 10 mm were used throughout. Baseline correction with physiological sodium chloride solution was performed on the spectrophotometer before analysis of samples. Between the scanning of samples the quartz cells were washed with de-ionised water and acetone and dried with air.

The spectral window used was determined in the following manner. Solutions of lidocaine in physiological sodium chloride were prepared at different concentrations. The solutions were scanned in the spectrophotometer and those which gave a spectrum with a maximum absorbance of around 2.5 were used further. With higher absorbance there is no longer a linear relationship between absorbance and concentration. The absorbance of lidocaine is in the wavelength region 190–290 nm (Fig. 1). However, as stray light is high in the lowest wavelength region, it was decided to reject the absorbance below 245 nm. Since lidocaine has no absorbance above 290 nm, the spectral window chosen was 245–290 nm. In this wavelength region lidocaine shows a characteristic shape of the absorbance spectra (Fig. 1).

Initial analysis of the five local anaestetics showed that four of these compounds had very similar UV spectra (Fig. 3). In order to gain as high resolution as possible from the spectra to be able to distinguish them, the minimum bandwidth of the instrument, 0.2 nm, was used throughout the study.

HPLC experiments were carried out using a conventional HPLC system consisting of the following components:, Shimadzu LC-10AD pump, HP G1313A autosampler with an injection volume of 20 μl and Shimadzu SPD-10A UV-Vis detector. The column was μBondapack C_{18} (150 × 3.9 mm id), the flow rate was 1.4 ml min^{-1} and the detector wavelength was 240 nm. The mobile phase was acetonitrile–phosphate buffer (pH 8.0) (3 + 2). The eluent was de-gassed with helium before use. The determination of lidocaine was carried out by means of linear regression with an external standard. An HP Chemstation was used for data acquisition and evaluation.

For the weighing of substances for sample preparation, a Sartorius MC-1, calibrated balance, was used.

For multivariate calibration, the Umetrics software Simca-P v. 7.01 was used on a Pentium computer with 32 Mb of RAM. The different data pre-treatments were carried out with the built-in function in the software.

Reagents

All chemicals and solvents were either of analytical-reagent or spectroscopic grade. Acetonitrile (AcN) was of gradient grade from Merck (Darmstadt, Germany). 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 hydrogenphosphate with water (Milli-Q, Waters). Physiological sodium chloride

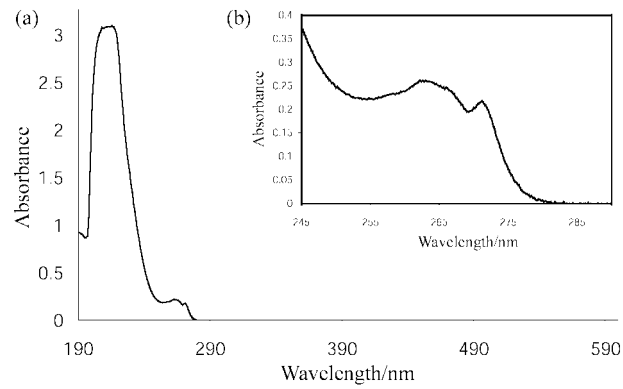

Fig. 1 (a) UV–Vis spectrum of lidocaine in the wavelength range 190–600 nm, bandwidth 0.2 nm. (b) Enlargement of the UV spectra in the range 245–290 nm, the region used in the study.

solution was made by dissolving 8.4 g of NaCl in 1000 ml of Milli-Q water. Lidocaine hydrochloride, 2,6-xylidine and the other four analgesic compounds used in the study (Fig. 2) were supplied by AstraZeneca Bulk Production (Södertälje, Sweden).

Since lidocaine is usually dissolved in physiological sodium chloride, the solutions used in the study were prepared with this solvent. The substances were weighed and dissolved in physiological sodium chloride solution, after which the solutions were further diluted with a Hamilton Microlab 1000 digital diluter to the concentration range 0.02–0.25 mg ml^{-1}. The sample solutions were prepared in this way to give different concentrations and mixtures (see Tables 1–6).

In the following text, the term 'accepted reference value' is used for the calculated concentrations of the sample solutions in the test sets.

Calibration model

The multivariate calibration model was constructed with PLS regression on a calibration set of OSC-treated spectra from 20 samples of known identity (lidocaine) and content. The calibration model consisted of 226 variables and 20 observations. The concentration range of lidocaine for the samples in the calibration set was 0.02–0.24 mg ml^{-1} (see Table 1).

The calibration model was validated with five external test sets (A–E) containing new samples of known identity and content. The prediction ability of the calibration model for the determination of the content of lidocaine was tested with external test sets A, C, D and E (see Tables 3-6). The identity determination was mainly tested with external test set B (see Table 2) since this test set contained other substances than lidocaine; however, the identity was also determined in the other four test sets. Comparisons between the reference method HPLC and the UV method were made in test sets D and E (see Tables 5 and 6).

The spectroscopic analyses of the calibration set and external test sets, A–C, were carried out in randomised order to minimise the effect of systematic errors.

Further descriptions of the external test sets A–E are as follows.

External test set A. Content and identity determination, 16 samples with different concentrations of lidocaine in the range 0.02–0.23 mg ml^{-1} (see Table 3).

External test set B. Identity determination, 26 samples. Four local anaesthetics, ropivacaine, bupivacaine, mepivacaine and prilocaine, and the main degradation product of lidocaine, 2,6-xylidine, in different mixtures (see Table 2).

External test set C. Repeatability, content and identity determination. Three samples containing lidocaine (0.039, 0.09 and 0.18 mg ml^{-1}) were scanned six times each and the results were compared in terms of content and identity determination (see Table 4).

External test set D. Comparison between HPLC and the UV method by identity and content determination with the two methods. Eight samples containing lidocaine (5 and 20 mg ml^{-1}) from an ongoing stability study. The samples were analysed with both HPLC and the UV method (see Table 5).

External test set E. Comparison between HPLC and the UV method over time by identity and content determination with the two methods. This test set contained five samples (control samples) of lidocaine in the concentration range 5–25 mg ml^{-1}. The samples were diluted and analysed on five separate occasions over a period of 5 months with the two methods (see Table 6). On this occasion the same samples were used in the two methods. For the HPLC analysis, new standard solutions were used each time.

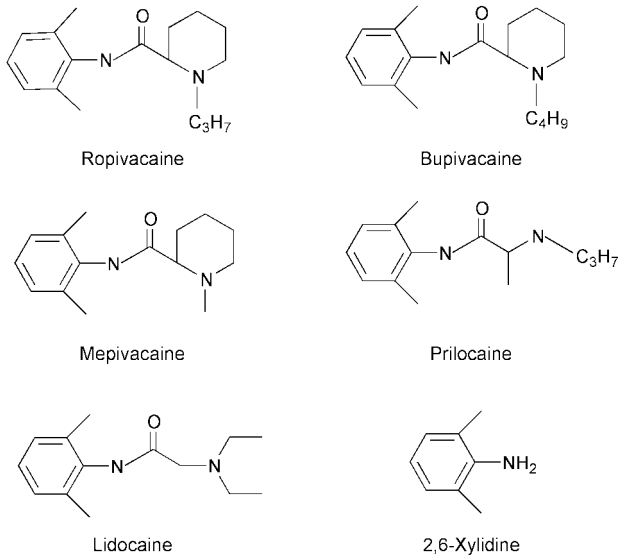

Fig. 2 Structures of the compounds used in the study. 2,6-Xylidine is the main degradation product of lidocaine.

Results and discussion

Calibration

Initial experiments with the UV method showed a systematic error when the same sample was analysed on two different occasions, the shape of the UV spectra looking the same but the absorbance being slightly different. Accordingly, the predicted content and the identity were also different. A drift in lamp intensity for the deuterium lamp was observed by means of the internal instrument test. In this test the relative energy of the two lamps was measured and the result is presented as a percentage of the energy compared with when the lamps were new (= 100%). In the initial experiments different values of the deuterium lamp energy were obtained when the energy was measured. This observation, together with fluctuating predictions of content and identity, led to the suspicion that this was caused by the drift in the deuterium lamp energy in spite of the double-beam instrument.

The deuterium lamp was changed and these problems were overcome in two additional steps. The first step was to randomise the order in which the samples were scanned. This was done together with the samples in the calibration set and the samples in external test sets A–C. In this way the systematic errors that could occur during the scanning time were eliminated and built into the model. The other step to avoid the systematic error was to apply OSC correction to every spectrum before the PLS model was created. The other data pre-treatments used did not work as well as the OSC correction based on the prediction of an external test set. Both SNV and MSC gave poor prediction results, with errors in both content and identity determination (results not shown). Hence the OSC correction was used in the rest of the study and no further investigations with the different types of data pre-treatments were made.

One OSC component was used and the model contained one PLS component. With the use of OSC there is probably an overestimate of the goodness of fit of the model. This is caused by the removal of portions of the X data that are linearly unrelated (orthogonal) to the response Y. The use of the external test sets shows that the predictability of identity and content of the calibration model is accurate (Tables 2–6).

Two types of scaling of the data were tested, unit variance and centring. Mean centring clearly gave the best results in terms of the calibration model and predictions (results not shown). Consequently, the final choice of pre-treatment was

first to apply OSC to all spectra and then to mean centre the data.

Identity determination

As can be seen in Table 1, a small variation between the DmodX values of the samples in the calibration set can be observed. This is probably due to small random errors in the experimental procedure of the analysis.

In Table 2, the results of the identity determination of external test set B can be seen. The prediction ability of the calibration model was tested with solutions of the local anaesthetics bupivacaine, ropivacaine, mepivacaine and prilocaine and the main degradation product of lidocaine, 2,6-xylidine. Different concentrations and mixtures of these compounds were analysed. All of the samples in external test set B have higher DmodXPS values than the samples in the calibration set, which means that these samples do not belong to the lidocaine class and therefore contain something else. The level of the DmodXPS value varies with the type of substance and its concentration. As can be seen in Table 2, prilocaine and 2,6-xylidine have the highest DmodXPS values. This indicates that these substances are most dissimilar to the lidocaine UV spectra. Furthermore, mixtures of prilocaine or 2,6-xylidine with lidocaine gave high DmodXPS values. This corresponds to the appearance of the UV spectra of prilocaine and 2,6-xylidine (Fig. 3), which are different to the other four local anaesthetics.

Table 1 Concentrations and DmodX values of the samples in the calibration set

Sample No.	Concentration/ mg ml^{-1}	DmodX
1	0.018	0.8
2	0.024	0.8
3	0.031	0.8
4	0.038	0.7
5	0.055	0.8
6	0.061	0.8
7	0.074	0.8
8	0.082	0.9
9	0.092	1.1
10	0.102	1.0
11	0.015	1.3
12	0.128	0.7
13	0.131	1.1
14	0.150	1.3
15	0.160	1.2
16	0.164	1.3
17	0.184	0.9
18	0.188	1.1
19	0.205	1.0
20	0.235	1.2

Table 2 Prediction results of the identity determination for the samples in test set B. Since the DmodXPS values are much higher than all of the samples in the calibration set, the identity is negative for all 26 samples

Sample No.	Content	Concentration/ mg ml^{-1}	DmodXPS
1	Bupivacaine	0.16	4.5
2	Bupivacaine	0.2	6.4
3	Bupivacaine	0.32	9.5
4	Ropivacaine	0.16	5.1
5	Ropivacaine	0.2	6.8
6	Ropivacaine	0.32	11.1
7	Mepivacaine	0.16	4.3
8	Mepivacaine	0.2	5.0
9	Mepivacaine	0.32	8.7
10	Prilocaine	0.08	97.3
11	Prilocaine	0/09	117.1
12	Prilocaine	0.16	196.7
13	Prilocaine	0.2	242.0
14	Prilocaine	0.32	369.1
15	2,6-Xylidine	0.16	64.6
16	2,6-Xylidine	0.2	76.9
17	2,6-Xylidine	0.32	112.8
18	Lidocaine/bupivacaine	0.1/0.1	2.9
19	Lidocaine/ropivacaine	0.1/0.1	3.6
20	Lidocaine/mepivacaine	0.1/0.1	3.0
21	Lidocaine/prilocaine	0.1/0.1	123.0
22	Bupi/Ropi/Mepi/Prilo/Lido[a]	0.32/0.35	102.2
23	Lidocaine/2,6-xylidine	0.027/0.01	16.9
24	Lidocaine/2,6-xylidine	0.11/0.04	27.4
25	Lidocaine/2,6-xylidine	0.11/0.085	42.0
26	Lidocaine/2,6-xylidine	0.11/0.17	65.8

[a] Bupivacaine/ropivacaine/mepivacaine/prilocaine/lidocaine.

From the results in Table 2 it is also possible to see that the DmodXPS value increases with increase in the concentration of the different compounds. This is probably due to the fact that the range of the calibration model is about 0.02–0.2 mg ml^{-1} of lidocaine. Higher concentrations of lidocaine or other compounds fall outside this range both in terms of their UV spectra and accordingly also their DmodXPS values. Of the mixtures of lidocaine and another local anaesthetic (samples 18–21 in Table 2), the mixtures of bupivacaine or mepivacaine with lidocaine seem to be the ones most like pure lidocaine. All of the other test sets contained only lidocaine solutions, and in all of these the DModXPS values were in the same range as in the samples in the calibration set.

Mepivacaine is the local anaesthetic most similar to lidocaine according to the DmodXPS values in Table 2. This is difficult to see in the spectra in Fig. 3. Here lidocaine, bupivacaine, ropivacaine and mepivacaine look almost the same. With the use of multivariate projections, however, the latter spectra contain sufficient information to distinguish them from that of lidocaine.

In classification there is a possible risk of obtaining a false positive result, *i.e.*, in this study a sample is classified as lidocaine but is, in fact, something else. However, this was not shown in the results obtained in the study. With HPLC there is also a risk of obtaining false positives, especially when only one wavelength is used for detection.

The bandwidth used in this study, 0.2 nm, was chosen to obtain as high a resolution as possible from the instrument. No further investigations into how an increase in bandwidth affects the classification ability have yet been performed. From the results obtained, it has been shown that with a bandwidth of 0.2 nm it is possible to distinguish the different local anaesthetics even though their UV spectra are very similar. With a bandwidth > 0.2 nm the resolution is reduced, which can lead to loss of information and poorer classification ability. However, it is reasonable to assume that the classification should work also with a bandwidth of 1 nm, but this has not been sufficiently investigated.

Content determination

The determination of content with PLS regression is done with the same calibration model as the identity determination.

In external test set A (Table 3), 16 samples with a known content of lidocaine were used to verify the prediction ability for content of the calibration model in the concentration range used (0.02–0.2 mg ml^{-1}). The prediction ability was within $< 1\%$ of the accepted reference value for all of the samples except the first two. One explanation for this may be that at low concentrations the influence of noise increases. The root mean square error of prediction (RMSEP)[12] was 0.0007. The identity for all of the samples in external test set A was positive.

In external test set C (Table 4), the repeatability was tested in terms of both content and identity determination. Three samples

containing different concentrations (0.039, 0.09 and 0.18 mg ml^{-1}) of lidocaine were scanned with the spectrophotometer six times each. The results show that the repeatability for content determination had a relative standard deviation (RSD) below 0.5% for all of the samples. In the HPLC reference method the RSD for six injections should be less than 1%. The RMSEP for this test set was 0.0003. The result of the identity determination was a positive identity for all of the samples.

In Table 5 the prediction results for the samples in external test set D can be seen. This test set contained eight samples from an ongoing stability study of lidocaine. The samples were analysed both with the UV method and with the HPLC reference method. The results show some small differences between the content determinations with the two methods. However, it is difficult from these results to separate the performances of the two methods, since the concentration varies within the given specification.

Table 6 contains the prediction results of content determination for the samples in external test set E, which was a long-term comparison between HPLC and the UV method. This test set contained five standard samples with a known content of lidocaine in the concentration range 5–25 mg ml^{-1}. Over a period of 5 months the samples were diluted from a standard solution and analysed with the two methods once every month. The reason for doing this was to investigate whether or not the systematic error seen in the initial experiments would influence the predictions of the model over time and also to obtain a comparison between the two methods, analysing the same samples of known content and identity several times. The results show that the two methods have comparable results in terms of content determination, although the mean of the difference between the accepted reference value and the predicted value is slightly lower for the UV method.

Estimate of analysis time

One of the major advantages of using this type of method with multivariate calibration coupled with spectroscopic techniques is its high speed. Clearly the time taken to scan a UV spectrum

Table 3 Prediction results of content and identity determination for the samples in external test set A. The absolute values (not shown) for both known concentration and predicted concentration were used for the calculation of the percentage difference between the accepted reference value and the predicted value (Difference). The DModXPS values are in the same range as the samples in the calibration set, indicating that the identity is positive

Sample No.	Accepted reference value/ mg ml^{-1}	Predicted concentration[a]/ mg ml^{-1}	Difference (%)	DmodXPS
1	0.023	0.022	4.3	1.0
2	0.03	0.03	1.9	0.8
3	0.038	0.038	< 1	0.7
4	0.047	0.048	< 1	0.9
5	0.056	0.056	< 1	0.8
6	0.068	0.067	< 1	0.8
7	0.077	0.077	< 1	0.7
8	0.09	0.09	< 1	1.0
9	0.095	0.094	< 1	0.8
10	0.113	0.112	< 1	0.7
11	0.127	0.126	< 1	1.1
12	0.141	0.141	< 1	1.1
13	0.159	0.158	< 1	1.0
14	0.180	0.180	< 1	1.2
15	0.198	0.198	< 1	1.2
16	0.225	0.225	< 1	1.2

[a] RMSEP: 0.0007.

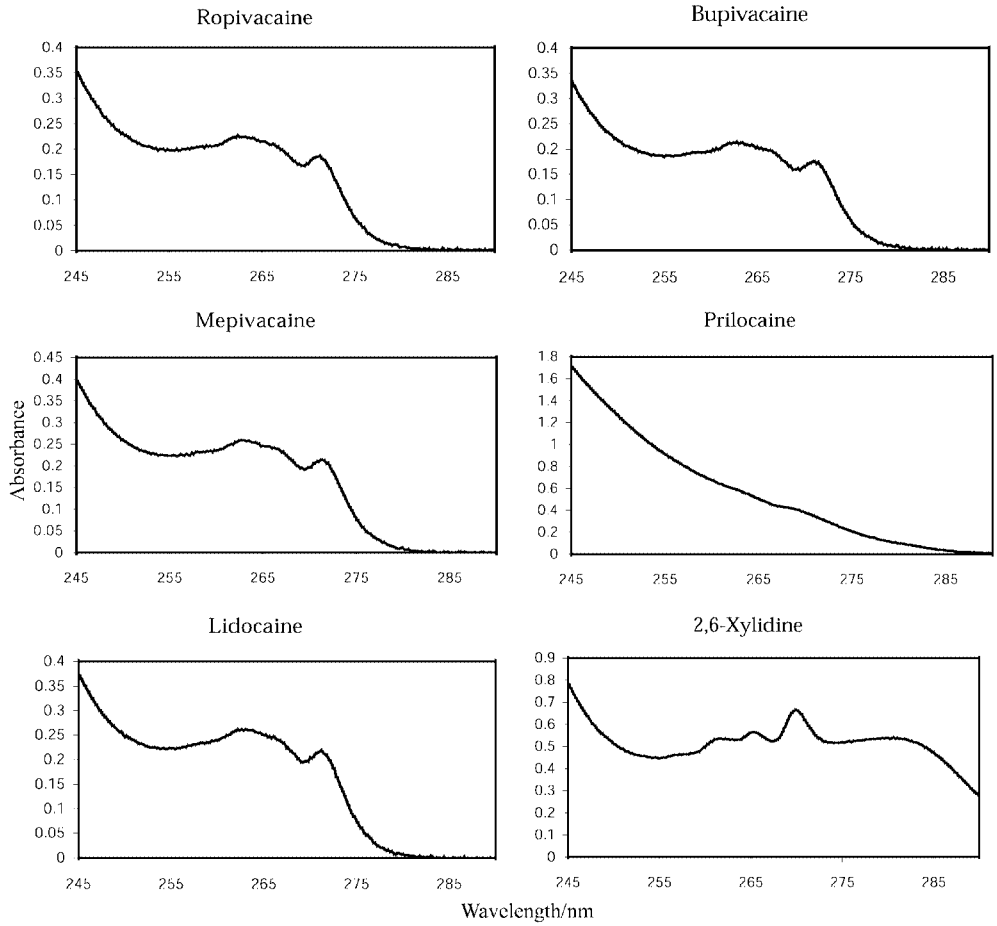

Fig. 3 UV spectra (245–290 nm) of the compounds in external test set B, dissolved in physiological sodium chloride solution. The concentration is about 0.16 mg ml^{-1} for all of the compounds.

of a sample is less than that to perform chromatography for separation and identification with external standards.

The HPLC method used in this study had a chromatographic run time of 5 min per sample. This is fairly fast for an HPLC method, although compared with the time spent for taking a UV spectrum in the range 245–290 nm it is rather long. Depending on the bandwidth, the time of analysis for the spectrophotometer used in this study was between 0.2 and 2.5 min per sample, where a bandwidth of 0.2 nm gave a time of analysis of 2.5 min.

An estimate of the total analysis time for an analysis of five samples with the two methods is given in Table 7. The stages of analysis are as follows:

(0) Construction of the calibration model is only done once, when the UV method is constructed.

(1) Preparation for analysis by the HPLC method involves preparing the mobile phase, weighing the working standard and preparing the standard solutions, diluting the samples and preparing the instrument. For the UV method, it involves diluting the samples. It is assumed that the spectrophotometer has been turned on for at least 1 h so that the instrument conditions are stable.

Table 4 Prediction results of content and identity determination for the samples in external test set C. The absolute values (not shown) for both accepted reference value and predicted concentration were used for the calculation of relative standard deviation (RSD) of the six predictions for each of the three samples. The DModXPS values are in the same range as the samples in the calibration set, indicating that the identity is positive

Sample No.	Accepted reference value/ mg ml^{-1}	Predicted concentration[a]/ mg ml^{-1}	Mean of predicted concentration/ mg ml^{-1}	RSD (conc.) (%)	DmodXPS
1a	0.039	0.0383	0.0385	0.3	0.7
1b		0.0386			0.7
1c		0.0385			0.6
1d		0.0385			0.7
1e		0.0383			0.7
1f		0.0384			0.6
2a	0.09	0.0897	0.0898	0.08	1.0
2b		0.0897			1.0
2c		0.898			0.9
2d		0.0897			1.0
2e		0.0899			0.9
2f		0.0897			0.9
3a	0.18	0.179	0.179	0.05	1.2
3b		0.179			1.2
3c		0.179			1.2
3d		0.179			1.1
3e		0.179			1.1
3f		0.179			1.2

[a] RMSEP: 0.0003.

Table 5 Prediction results of content determination for the samples in external test set D. The identity was positive for all samples with both methods

Sample No.	Labelled concentration/ mg ml^{-1}	HPLC predicted concentration/ mg ml^{-1}	Difference from labelled value (%)	UV method predicted concentration/ mg ml^{-1}	Difference from labelled value (%)
1	5.0	5.03	0.6	5.05	1.0
2	5.0	5.03	0.6	5.02	0.4
3	5.0	4.96	0.8	5.0	0
4	5.0	4.95	1.0	4.96	0.8
5	20.0	20.3	1.5	20.3	1.5
6	20.0	20.2	1.0	20.4	2.0
7	20.0	20.1	0.5	20.2	1.0
8	20.0	20.1	0.5	20.2	1.0

(2) Analysis of sample relates to the actual time taken for the instrument to analyse the five samples, which for the HPLC method also means the external standards.

(3) Evaluation of the results is the calculation and prediction of content and identity and the completion of the analysis. For the HPLC method, this means re-integration of all chromatograms and taking out the report file from the chromatographic data system. For the UV method, this means transferring the digitised UV spectra from the UV software to the calibration software and performing the content and identity determination.

In the time estimates in Table 7 it is clear that when the number of analyses increases the time saved by using the UV method also increases dramatically. The time used at the

Table 6 Prediction results of content determination for the samples in external test set E. The identity was positive for all of the samples with both methods

Control Sample No.	Accepted reference value/ mg ml^{-1}	HPLC predicted concentration/ mg ml^{-1}	Difference from accepted reference value (%)	UV method predicted concentration/ mg ml^{-1}	Difference from accepted reference value (%)
1	5.07	5.09	0.4	5.06	0.2
2	10.03	10.07	0.4	9.94	0.9
3	15.03	15.14	0.7	15.07	0.3
4	20.07	20.11	0.2	20.01	0.3
5	25.05	25.5	1.8	25.3	1.0
1	5.07	5.2	2.6	5.06	0.2
2	10.03	10.15	1.2	10.0	0.3
3	15.03	15.22	1.2	14.9	0.9
4	20.07	20.49	2.1	20.0	0.3
5	25.05	25.81	3.0	24.9	0.6
1	5.07	5.11	0.8	5.06	0.2
2	10.03	10.14	1.1	9.95	0.8
3	15.03	15.32	1.9	14.92	0.7
4	20.07	20.32	1.3	19.79	1.4
5	25.05	25.24	0.8	25.01	0.2
1	5.07	5.12	1.1	5.05	0.4
2	10.03	10.22	1.9	9.98	0.5
3	15.03	15.13	0.7	14.86	1.1
4	20.07	20.07	0.1	19.92	0.7
5	25.05	25.10	0.2	24.85	0.8
1	5.07	5.10	0.7	5.03	0.8
2	10.03	10.13	1.0	9.94	0.9
3	15.03	15.11	0.5	14.86	1.1
4	20.07	20.08	0.05	19.86	1.1
5	25.05	25.45	1.6	24.87	0.7
		Mean:	1.1	Mean:	0.7
		s:	0.8	*s*:	0.4

Table 7 Estimation of the total analysis time for determination of content and identity for five samples of lidocaine with the two methods. The estimated times are approximations

	Time of analysis/h	
Stage of analysis	HPLC	UV method
(0) Construction of calibration model	–	5
(1) Preparation of analysis	3	0.2
(2) Analysis of samples	1	0.3
(3) Evaluation of results	1	1
Total time of analysis of 5 samples	5	6.5
	Total time of analysis/h	
No. of analyses of 5 samples	HPLC	UV method
1	5	6.5
2	10	8
5	25	12.5
10	50	20
25	125	42.5

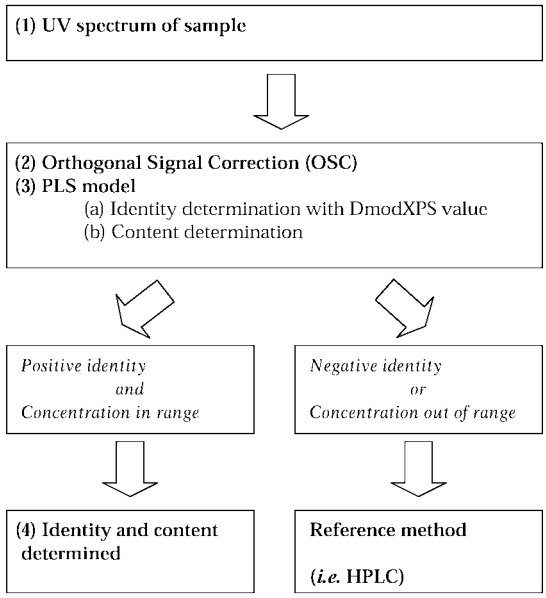

Fig. 4 Schematic diagram of the procedure for the identity and content determination with the UV method. In the case of negative identity or if the concentration is out of range, the HPLC reference method is used.

beginning of the UV method for construction of the calibration model is clearly well spent if one is going to analyse many samples. For the UV method item (2), the time of analysis by the instrument can probably be decreased even more with the use of automation. In this study, a manual spectrophotometer was used with ordinary open-topped rectangular quartz cells that were filled and cleaned manually. However, using a more automated approach, *e.g.*, with a flow-through cell, the estimated time in item (2) would decrease even more.

Practical considerations

The practical procedure for an analysis of a new sample with the UV method is summarised in Fig. 4. The calibration model is assumed to have been made.

After scanning, the spectrum is transferred in digitised form to the calibration software, where the spectra are treated with OSC. The data for the sample are then used in the calibration model for the determination of identity and content. If the identity is positive and the concentration is in the calibrated range, the analysis is concluded and content and identity have been determined. However, in the case of negative identity and/or if the concentration is out of the calibrated range, the reference analytical method, HPLC, is used for evaluation.

In this way HPLC is included in the method. A final answer is not obtained from a sample with negative identity until the HPLC measurement has been made.

It is advisable when using these types of analytical methods also to analyse control samples of known identity and content every time the method is used. This is a convenient way to check that the method is working as it should and to provide a day-to-day inspection of the behaviour of the instrument.

Conclusion

This work shows that the multivariate UV method with OSC works well with the tested compounds even though the UV spectra of lidocaine, bupivacaine, ropivacaine and mepivacaine are very similar. With the SIMCA method and the DmodX values there seems to be sufficient information to allow the different compounds to be distinguished from each other.

In terms of accuracy, precision and repeatability, the proposed method is comparable to the reference method, HPLC. With respect of analytical time, the proposed method is much faster. The main advantage, therefore, of this new method over HPLC is the much shorter time of analysis.

Acknowledgements

The authors thank Dr. Mats Josefson at AstraZeneca R&D Mölndal for carefully reading the manuscript and Dr. Anders Sparén at AstraZeneca Tablet Production for providing information on the calculation of distance to model.

References

1 *ASTM Standard E 1655-94*$^{\varepsilon 1}$, American Society for Testing and Materials, Philadelphia, 1995.
2 R. D. Bautista, A. I. Jiménez, F. Jiménez and J. J. Arias, *J. Pharm. Biomed. Anal.*, 1996, **15**, 183.
3 C. Demir and R. G. Brereton, *Analyst*, 1998, **123**, 181.
4 E. Engström and B. Karlberg, *J. Chemom.*,1996, **10**, 509.
5 T. Kappes, G. López-Cueto, J. F. Rodriguez-Medina and C. Ubide, *Analyst*, 1998, **123**, 2071.
6 P. Gratteri and G. Cruciani, *Analyst*, 1999, **124**, 1683.
7 M. M. Sena, J. C. B. Fernandes, L. Rover, Jr., R. J. Poppi and L. T. Kubota, *Anal. Chim. Acta*, 2000, **409**, 159.
8 K. Wrobel , K. Wrobel, P. L. Lopez-d-Alba and L. Lopez-Martinez, *Anal Lett.*, 1997, **30**, 717.
9 P. Geladi and B. Kowalski, *Anal. Chim. Acta*, 1986, **185**, 1.
10 M. Sjöström and S. Wold, *Anal. Chim. Acta*, 1983, **150**, 61.
11 R. G. Brereton, *Analyst*, 2000, **125**, 2125.
12 H. Martens and T. Naes, *Multivariate Calibration*, Wiley, New York, 1989.
13 W. J. Dunn and S. Wold, *Methods Princ. Med. Chem.*, 1995, **2**, 179.
14 R. De Maesschalck, A. Candolfi, D. L. Massart and S. Heuerding, *Chemom. Intell. Lab. Syst.*, 1999, **47**, 65.
15 B. Mertens, M. Thompson and T. Fearn, *Analyst*, 1994, **119**, 2777.
16 O. M. Kvalheim, K. Oygard and O. Grahl-Nielsen, *Anal. Chim. Acta*, 1983, **150**, 145.
17 *Users Guide to Simca-P 7.01*, Umetrics, Umeå, Sweden, 2000.
18 S. Wold and M. Sjöström, *ACS Symp. Ser.*, 1977, **52**, 243.
19 C. Albano, G. Blomqvist, D. Coomans, W. J. Dunn, III, U. Edlund, B. Eliasson, S. Hellberg, E. Johansson, B. Nordén, D. Johnels, M. Sjöström, B. Söderström, H. Wold and S. Wold, in *Proceedings of the Symposium on Applied Statistics, Copenhagen, January 22, 1981*.
20 R. J. Barnes, M. S. Dhanoa and S. J. Lister, *Appl. Spectrosc.*, 1989, **43**, 772.
21 S. Wold, H. Antti, F. Lindgren and J. Öhman, *Chemom. Intell. Lab. Syst.*, 1998, **44**, 175.