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ORIGINAL RESEARCH

Attempt to separate the fluorescence spectra of adrenaline and noradrenaline using chemometrics

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ABSTRACT: An investigation was conducted on whether the fluorescence spectra of the very similar catecholamines adrenaline and noradrenaline could be separated using chemometric methods. The fluorescence landscapes (several excitation and emission spectra were measured) of two data sets with respectively 16 and 6 samples were measured, the smaller data set with higher resolution and i.e. precision. The samples were artificial urine (pH $\simeq$ 3) spiked with the catecholamines in the concentration ranges 40–1200 nmol/L and 5.5–18 μmol/L, respectively. Unfold partial least squares regression (Unfold-PLSR) on the larger data set and parallel factor analysis (PARAFAC) of the six samples of the smaller set showed that there was no difference between the fluorescence landscapes of adrenaline and noradrenaline. It can be concluded that chemometric separation of adrenaline and noradrenaline is not obtainable using this type of fluorescence measurement. Raman scatter, which overlaps the catecholamine spectra, was shown not to have any influence on the models calculated. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: catecholamines; fluorescence; chemometrics; PARAFAC; urine

INTRODUCTION

Adrenaline and noradrenaline are hormones related to the sympathetic medullary, and these hormones (along with cortisol) are reported to be particularly sensitive to mental stress (1, 2). Urinary catecholamine excretion is a measure of acute stress, and has been used as such in several occupational health investigations, see e.g. (3–5). Development of a faster and cheaper method for determining adrenaline and noradrenaline in urine is necessary if these biomarkers are to be used extensively in the investigation and prevention of stress in the working environment. Fluorescence spectrometry is a fast, inexpensive and sensitive analytical method, which makes it an attractive alternative detection method, as the catecholamines are fluorescent.

The fluorophore of adrenaline and noradrenaline[†] is the same, namely catechol ($C_6H_6O_2$), and the compounds are expected to have very similar fluorescence properties. The difference between adrenaline and noradrenaline is a methyl group, which makes adrenaline a secondary amine, while noradrenaline is primary (see Fig. 1). It is important that catecholamine solutions are kept at acidic pH values, where the amino groups are protonated, as the free catecholamines deteriorate in neutral and alkali media (6, 7), due to oxidation of the phenolic groups. The compounds only have slightly different dissociation constants (pK_a adrenaline, 8.7, 10.2 and ca. 12; pK_a noradrenaline, 8.6, 9.8 and ca. 12) (8). It has not been possible to find consistent information on how the fluorescence properties of the catecholamines vary with the pH value. However, preliminary experiments have shown that the excitation and emission maxima do not seem to change as a function of pH as long as the pH is below 7. It has been further observed that the native fluorescence vanishes as soon as the pH in the solution is slightly alkaline (due to deprotonization). Several authors, e.g. (9–11), have described the formation of lutines (3,5,6-trihydroxyindole-derivates) in strongly alkaline media after oxidation of the catecholamines to adreno- and noradrenochrome, respectively. Oxidation is obtainable if dissolved oxygen is present in the sample, but quantitative oxidation is attained using an oxidation agent such as hexacyanoferrate(III). The fluorescence from the lutines rather quickly fades out unless an antioxidant is present. The influence of pH will not be

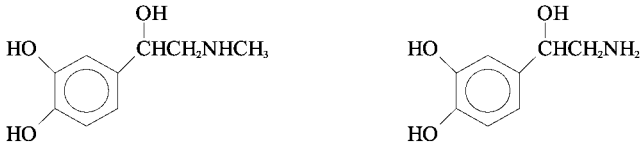

Figure 1. Structural formula for adrenaline (left) and noradrenaline (right).

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[†]Adrenaline's, systematic name is: D-1-[3,4-dihydroxyphenyl]-2-methyl-aminoethanole and noradrenaline's: is D-1-[3,4-dihydroxyphenyl]-2-aminoethanole.

further investigated in this work because the main purpose here is the potential development of a method in which no or little sample preparation is necessary. An analytical method in which physical and/or chemical separation of the catecholamines is replaced with a chemometric separation of the native catecholamine fluorescence spectra would save time and reagents compared to previously published methods, as fewer analytical procedures would be involved. Hence, for the experiments described below, a pH is chosen at which the catecholamines are stable.

At present, high performance liquid chromatography (HPLC) methods are typically used for the separation of adrenaline and noradrenaline followed by quantification, using, for example, fluorescence or electrochemical detection [see e.g. (12–15)]. A method using a cation-exchange column, derivatization to the trihydroxyindole derivates, followed by separation by second derivative synchronous fluorescence spectroscopy was published several years ago (16) and recently improved (17). Several other types of analytical methods, such as radioimmunoassay (RIA) (18), enzyme-linked immunosorbent assay (ELISA) (19) and capillary electrophoresis (20) have been developed for catecholamine analysis.

Parallel factor analysis (PARAFAC) (21–23) of composite fluorescence data has previously been applied to biological matrices, e.g. sugar (24), for quantitation and resolution of mixtures, with good results. The first step in developing an analytical procedure for adrenaline and noradrenaline in urine samples using fluorescence and chemometrics is to find out whether the fluorescence spectra of the catecholamines actually can be resolved using chemometric methods, i.e. whether there are detectable differences in the fluorescence signals between the two compounds.

The aims of the present study are: first to investigate whether the fluorescence spectra of the catecholamines adrenaline and noradrenaline can be implicitly separated by using appropriate multivariate calibration methods; specifically unfold partial least squares regression (Unfold-PLSR) (25) or PARAFAC (21, 23); second, to attempt to quantify the catecholamines; and third, to discuss the influence of scattering phenomena on the spectra measured.

MATERIALS AND METHODS

Instrumentation

The samples were measured on a Perkin-Elmer LS50B Luminescence Spectrometer with a 10 mm quartz cuvette. FL Data Manager, version 3 (Instrument Control, version 4) was used for data collection; the fluorescence landscapes were measured using an OBEY programme (OBEY version 3.5, Perkin-Elmer). The spectra were imported to MATLAB®, version 5.3, using INCA software (Andersson, CA, MATLAB file, http://www.models.kvl.dk, version 1.4). The N-way Toolbox, also downloaded from http://www.models.kvl.dk, was used for calculations. The Unscrambler®, version 7.5, was used for the principal component analysis (PCA) and Unfold-PLSR calculations.

Reagents and sample preparation

The following stock solutions were prepared. Chemicals were used as supplied, without further purification. 10 mg of the free bases of the catecholamines (Fluka; product numbers 74460 and 02252, or Sigma, product numbers E4250 and A7257) were each dissolved in 100 ml 1 mmol/L hydrochloric acid (Merck, 1.00318.1000). An artificial urine solution was prepared from sodium chloride (Bie & Berntsen, BBB23036), diamoniumhydrogenphosphate (Merck, 1.1207-500) and sulphuric acid (Merck, 1.00714.1000). The chemicals were dissolved in Milli-q-water (Millipore, Waters, Taastrup, DK) in levels similar to the concentrations in human urine. From the three stock solutions, artificial urine samples spiked with the catecholamines were prepared (see concentration ranges in Table 1).

Table 1. Experimental conditions for the two sample sets measured on a Perkin-Elmer LS 50B. Set 2 was measured with different scanning speeds on 2 different days

	Number of samples	Concentration range for total amount of catecholamines	Excitation and emission slit widths (nm)	Scanning speed (nm/min)	Wavelengths measured
Set 1	16*	40–1200 nmol/L	10	1500	λ_{ex}: 230–330 nm with steps of 5 nm, and 340–500 nm with steps of 10 nm λ_{em}: from (λ_{ex} + 10 nm) to (2 × λ_{ex} − 20 nm)
Set 2	6	5.5–18 μmol/L	3	60 30	λ_{ex}: 265–290 nm with steps of 1 nm λ_{em}: from (λ_{ex} + 5 nm) to 400 nm

* Five of them measured in duplicate, i.e. 21 measurements.
λ_{ex}, excitation wavelengths; λ_{em}, emission wavelengths.

Sample sets

To keep the set-up as simple as possible, artificial urine spiked with catecholamines was used instead of real urine samples. Using simple artificial urine was expected to minimize potential problems such as quenching and total absorption. Furthermore, if separation were not possible in these model systems, then separation of adrenaline and noradrenaline probably could not be expected in real urine samples. Hence, the result using artificial urine was assumed to provide a good initial basis for assessing the potential of using mathematical separation of the fluorescence signals.

Two sample sets were prepared: one with 16 samples (set 1) and one with six samples (set 2). All samples in each sample set were prepared from the same artificial urine solution. The pH value for set 1 is 3.01 and for set 2, 3.15. Both sample sets consisted of samples with adrenaline and noradrenaline separately and in mixtures. The purpose of having two sample sets was that one reflected the typical clinical situation, where there are low concentrations and little time to scan all samples at a low scan speed (i.e. high resolution). The smaller set suited the theoretical investigation of whether the fluorescence spectra of adrenaline and noradrenaline can be separated at all using optimal resolution conditions (higher concentrations and lower scan speed).

Measurements

Emission was automatically measured in steps of 0.5 nm, and other details are given in Table 1. It should be noticed that set 2 was measured with low scanning speed (60 nm/min on one day and 30 nm/min on another, to enhance sensitivity further), small slit widths and excitation steps. This provided high resolution, however at the cost of signal intensity due to the narrow slit widths. Therefore, the catecholamine concentrations had to be higher than the natural urine concentrations (which were in the nanomolar range), to get a satisfactory fluorescence signal (see Table 2). The excitation and emission peaks found in the spectra measured were in accordance with those reported in the literature. The excitation peaks were expected to be in the areas 205–210 nm and 275–285 nm and the emission peak in the range 310–340 nm (26–28). Usually the first excitation peak is too noisy to be of use, and also many other biological materials fluoresce in this spectral region, which makes the peak rather non-specific. Hence, only the second excitation peaks were used in this work. The excitation and emission ranges were chosen sufficiently wide to include the second emission peak (see Table 1). The size of the fluorescence landscapes was reduced to include only the most interesting wavelengths before fitting the models, which will be described in detail later. All samples were measured at room temperature (for set 1, 25.5°C; for set 2, 22.5°C on both days), which remained constant within 1°C during the measurement of each set.

Chemometric analysis

PCA and Unfold-PLSR. Principal component analysis (PCA) (25) was applied to identify possible outliers and to see how many principal components were needed to express the information in the variables (the fluorescence spectra). This gave an indication of the number of factors expected to be adequate for the PARAFAC and Unfold partial least squares regression models. Multivariate calibration (Unfold-PLSR) (25, 29) was employed to calculate calibration models, for prediction of the catecholamine concentrations from the fluorescence data.

PARAFAC. Fluorescence intensity measurements can, ideally, be described (30) as:

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \quad (1)$$

where x_{ijk} is the intensity measured in sample i at

Table 2. Catecholamine concentrations in set 2. The two columns to the right contain calibration results from one-component PARAFAC models where Raman scatter had been removed by subtracting a blank, and the spectra were corrected for interference from the other component (noradrenaline or adrenaline, respectively) prior to calculating the model. Sample 5 was used as a calibration sample. Quite good agreement with the actual concentrations was seen.

Sample No.	Concentration of adrenaline (μmol/L)	Concentration of noradrenaline (μmol/L)	Predicted values from PARAFAC one-component calibration for adrenaline (μmol/L). Mean value from 2 days ± SD	Predicted values from PARAFAC one component calibration for noradrenaline (μmol/L). Mean value from 2 days ± SD
1	2.5	5.0	2.4 ± 0.08	4.9 ± 0.08
2	7.0	9.0	6.2 ± 0.12	8.4 ± 0.04
3	9.0	0	8.2 ± 0.27	0 ± 0
4	0	12.0	0 ± 0	11.5 ± 0.17
5	1.5	4.0	1.5*	4.0*
6	11.0	7.0	9.6 ± 0.45	6.3 ± 0.26

* Calibration sample.

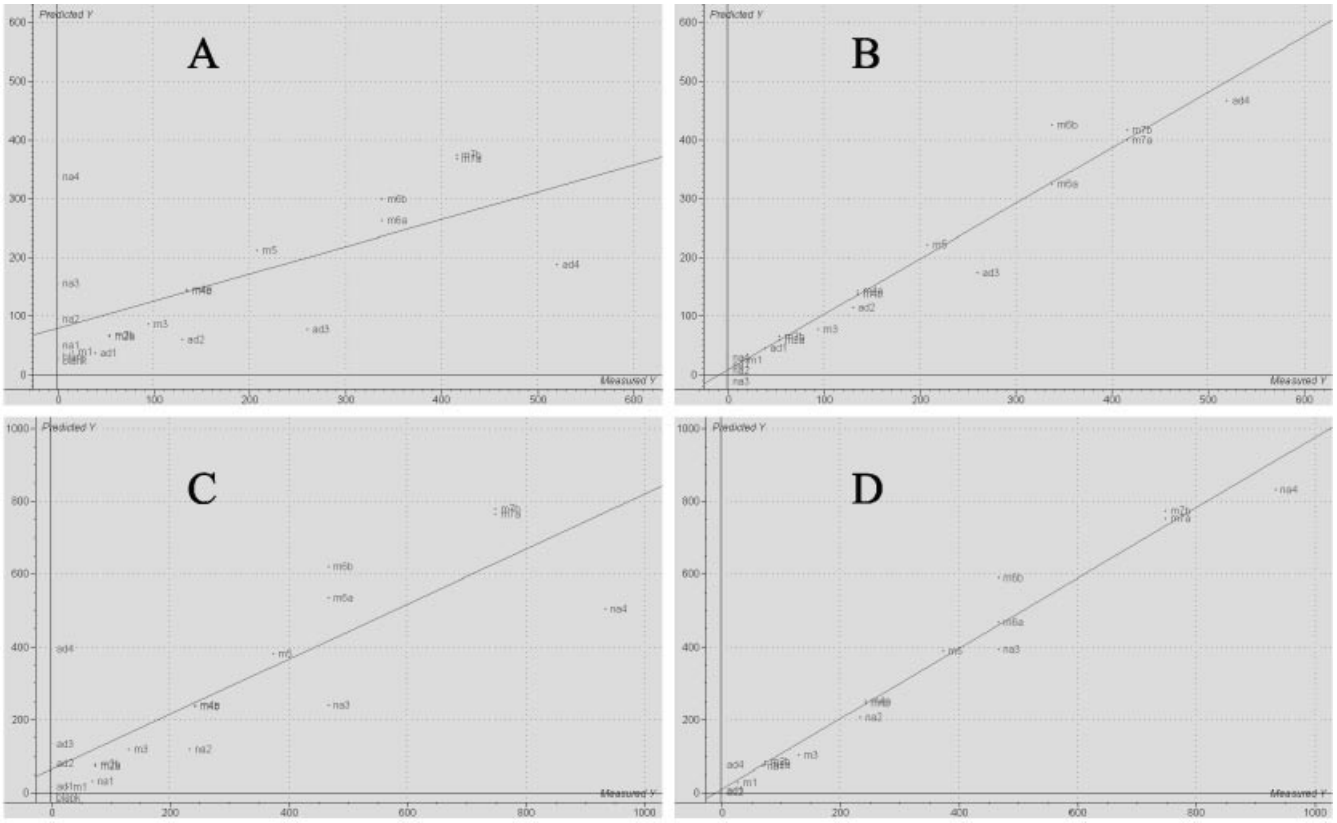

Figure 2. Predicted vs. measured plots for four one-component Unfold-PLS1 regressions with different *y* variables: top, adrenaline; bottom, noradrenaline. The uncorrected calibrations are shown on the left (A, C), the corrected ones on the right (B, D). The unit for all axes is nmol/L. Samples marked ad are pure adrenaline, na are pure noradrenaline and the m-samples are mixtures of the two (the eventual a and b after the number are replicates). For further details, see Table 3.

emission wavelength j and excitation wavelength k. This intensity is a sum of a small amount of noise (e_{ijk}) and the contribution from F fluorophores. For each fluorophore the contribution is independent of the contribution from other fluorophores when quenching, energy transfer, etc. is absent. The individual contribution from the fth fluorophore can be written as $a_{if}b_{jf}c_{kf}$, where a_{if} is linearly related to the concentration of the fth fluorophore in the ith sample. The parameter b_{if} is linearly related to the relative fluorescence intensity of the fth fluorophore at emission wavelength j, and c_{jk} is linearly related to the extinction coefficient of the fluorophore at excitation wavelength k. Hence the vector $\mathbf{b}_f$ with typical elements b_{jf} will hold the emission spectrum of analyte f, and correspondingly the vector $\mathbf{c}_f$ with typical elements c_{kf} will hold the excitation spectrum.

This model of fluorescence data is identical to the trilinear PARAFAC model originally suggested within psychometrics (21) but now widely used in chemometrics (22, 23).

A PARAFAC solution is essentially unique, meaning that ideally the true emission and excitation spectra of each compound in a mixture can be resolved, when the correct number of components/fluorophores, F, is chosen

Table 3. Leave-one-out cross-validation results for set 1 from four one-component Unfold-PLSR-1 calibrations. Results for both uncorrected and corrected spectra are shown. RMSECV, root mean squared error of cross-validation.

	Adrenaline (uncorrected)	Adrenaline (corrected)	Noradrenaline (uncorrected)	Noradrenaline (corrected)	Ideal value
Slope of $x = y$	0.46	0.95	0.76	0.97	1
Offset (nmol/L)	78.9	8.2	63.1	8.6	0
Correlation	0.66	0.98	0.85	0.99	1
RMSECV (nmol/L)	121.9	33.2	148.2	47.2	0
Bias (nmol/L)	−1.66	−0.68	1.33	−0.47	0

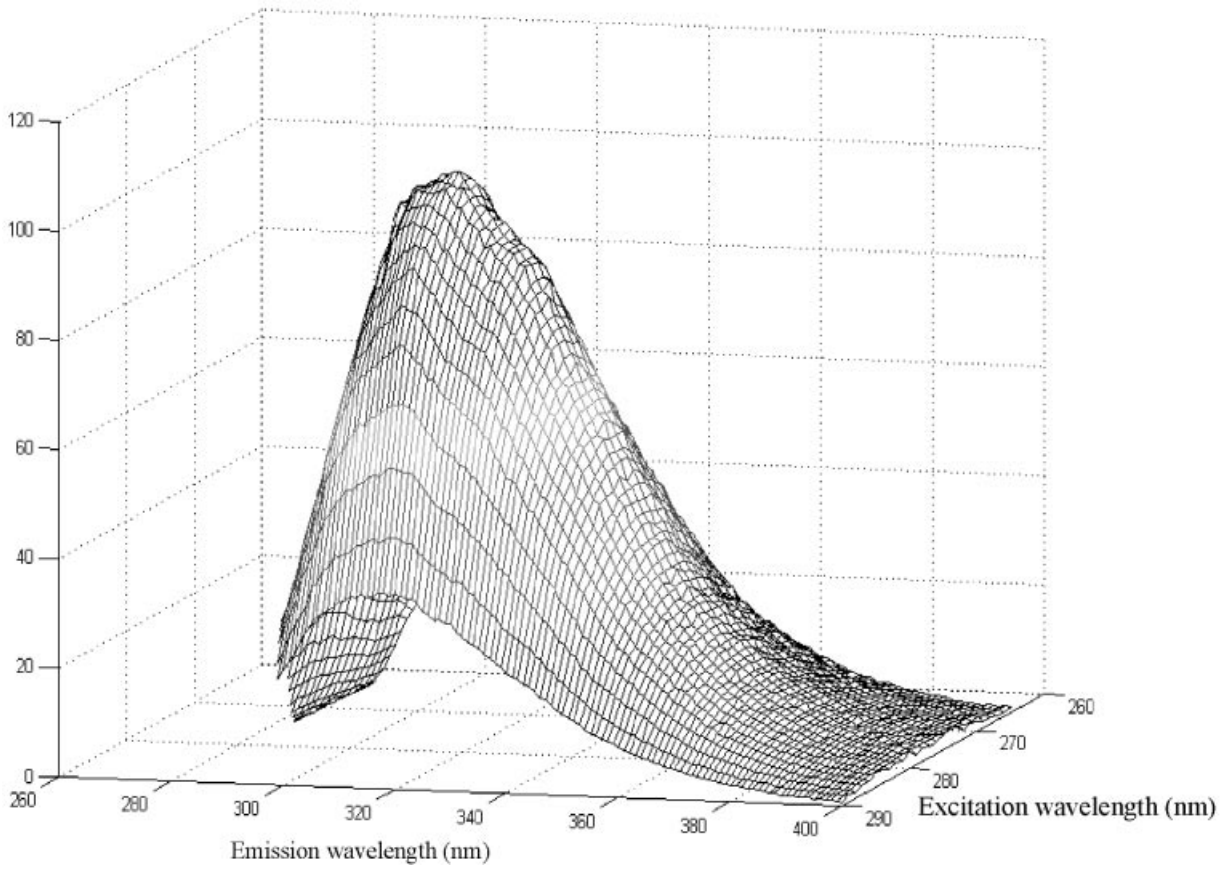

Figure 3. Fluorescence landscape of a typical sample (a mixture of adrenaline and noradrenaline) from set 2. Rayleigh scatter has been removed.

for the PARAFAC model. This requires that all excitation and emission spectra are linearly independent (i.e. no two are identical in shape) and that the concentration of the analytes varies independently. For these data, the concentrations are experimentally controlled to vary independently, so the critical aspect is whether the emission and excitation spectra of noradrenaline and adrenaline are identical. Using the PARAFAC model, even spectra of fluorophores that severely overlap can be separated; thus providing the means for direct quantitative and qualitative analysis of mixtures. If the nature of the data is not truly trilinear—e.g. scatter effects in fluorescence do not have a trilinear structure—the modelling can be disturbed. Sometimes, constraining the model can be helpful if the model parameters are difficult to estimate. Examples of constraints useful for spectrometric measurements are unimodality and, especially, non-negativity.

In the following, Unfold-PLSR and especially PARAFAC models will be used for assessing the similarity of adrenaline and noradrenaline in artificial urine, in order to provide evidence of whether the spectra of these components can be expected to be separated using an instrument of moderate to high resolution.

RESULTS AND DISCUSSION

Scatter interferences

The raw spectra contained two types of scatter interferences, Raman and Rayleigh (see e.g. Taylor, 1996; http://www.perkin-elmer.com/ai/ai.nsf/pages/flrtech.html) which were both of significant size. Raman scatter was mainly due to the solvent (water) in diluted solutions, while Rayleigh scatter was caused by the molecules in the solution. These scattering effects did not follow the PARAFAC model, and could incorrectly bias the estimated parameters (24).

The Rayleigh scatter was removed using the INCA software (Andersson, CA, MATLAB file, http://www.models.kyl.dk, version 1.4). INCA removes a band of wavelengths around the lines where the emission wavelength equals n times excitation wavelengths ($n = 1$ 0th order scatter and/or two 1st-order scatter). The bandwidth was chosen manually so as to manually optimize the removal of the banded scatter while maintaining the signal. The removal of the signal meant that the elements where the scatter occured were set to be missing. In the subsequent modelling of the data, these

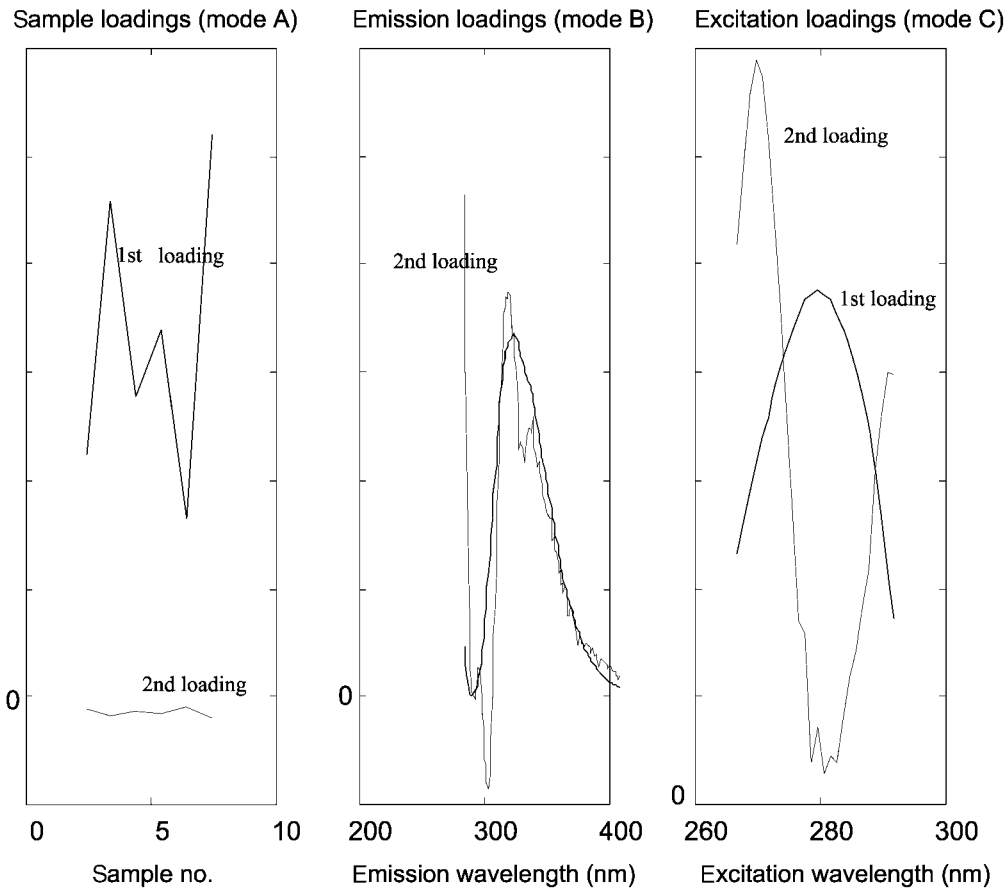

Figure 4. Loadings from a PARAFAC model two with components calculated for set 2. The first loadings in mode B and C are similar to catecholamine spectra, with peaks at approximately 315 nm and 277 nm, respectively. The scaling on the ordinate axes is arbitrary.

elements were then not used and hence did not influence the fitted parameters [see e.g. (23)]. The Raman scatter could not be removed from the spectra by setting the elements where it occurred to 'missing', as it coincided with the catecholamine peaks; at the excitation wavelength 275 nm, the emission peak was 310 nm (Taylor, 1996; http://www.perkin-elmer.com/ai/ai.nsf/pages/flrtech.html). Hence, the non-trilinear Raman scatter remained in the spectra during PARAFAC calculations. This was a potential problem but, as will be shown below, had no significant influence.

Analysis of set 1

The raw[†] spectra from the LS50B were transformed into the MATLAB format using INCA, at the same time removing 0th and 1st order Rayleigh scatter (bandwidths of 18 and 25 nm for 0th and 1st order scatter, respectively were found to be optimal), and selecting only every second emission wavelength measured (i.e. the resulting step size was 1 nm). Some of the wavelengths containing no catecholamine information were removed. Furthermore, all emission below excitation was also set to 'missing', because it did not follow the model in Eq. 1. The final data set had the dimensions: 21 concentrations × 90 emission wavelengths × 14 excitation wavelengths, the emission wavelengths in the range 285–375 nm and the excitation wavelengths 230–295 nm, and there were 8.3% missing values. A PCA of these data (with samples as rows and emission spectra at consecutive excitations as columns) showed no outliers and one principal component (as evidenced through leave-one-out cross-validation) was optimal and explained 98% of the information in the data. Two Unfold PLS-1 regressions were calculated using 16 cross-validation segments, with one sample per segment. A blank spectrum was subtracted from all spectra to reduce scatter interferences, before they were centered prior to PLS-calculations. The reference values for the two models were the noradrenaline and adrenaline concentration, respectively. The predicted vs. measured plots are shown on the left in Fig. 2 (A and C). One PLS component was optimal (showed the lowest root mean

[†]They were not smoothed or otherwise pre-treated.

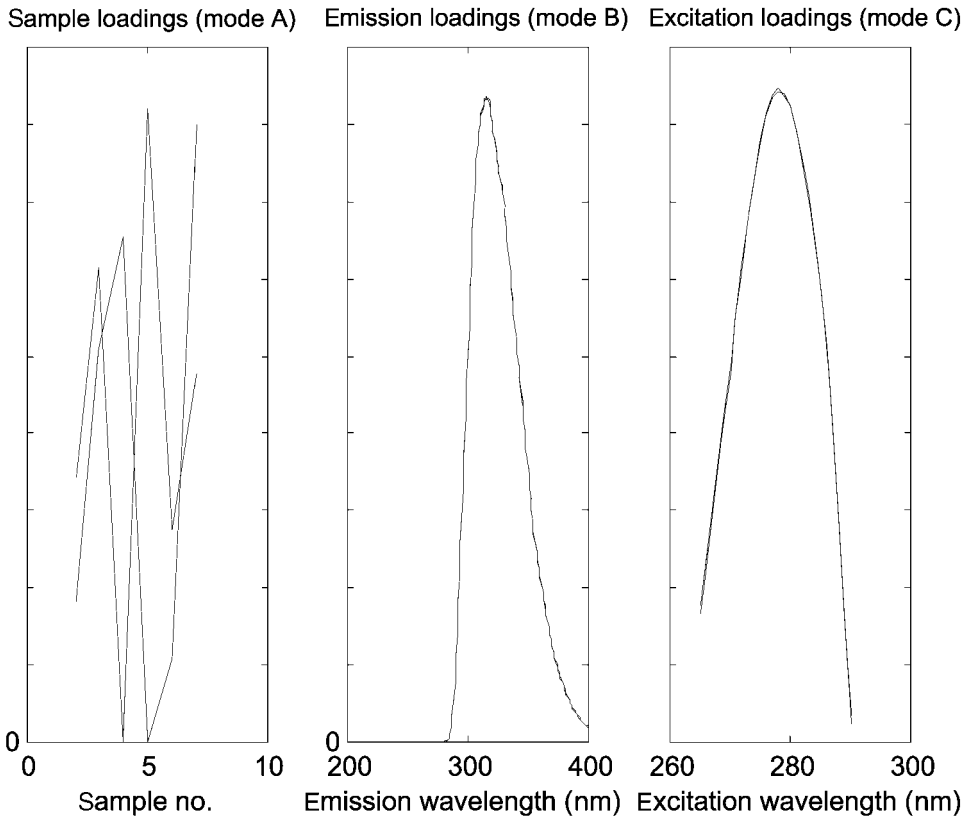

Figure 5. Loadings from a PARAFAC model two with components, non-negativity constraints, and restricted concentration mode calculated for set 2. The scaling on the ordinate axes is arbitrary.

squared error of cross-validation (RMSECV) etc.) for both models. RMSECV measures the cross-validation error in the same units as the original response variable and was calculated from the predictions obtained by leaving out one sample at a time and then predicting its concentration from a model fitted without the sample (25). It was clear from plot A and C in Fig. 2 that the models could not differentiate between adrenaline and noradrenaline. In the adrenaline plot (A) it was noteworthy that the noradrenaline samples were placed along the ordinate axes, because the model predicted a considerable adrenaline content in these samples, although the actual adrenaline concentration was zero. The same pattern was seen for adrenaline in the noradrenaline plot (C). Unit spectra for adrenaline and noradrenaline were calculated by dividing the spectra of pure samples with the matching concentration. It should be noted that noradrenaline showed weaker fluorescence intensity than adrenaline at the chosen pH value (31), hence the unit spectra also had slightly different intensities. The mean of the unit spectra of the three pure samples with the highest concentrations was the resulting unit spectrum for each of the two components. Prior to calculating the corrected Unfold PLS-1 regression for adrenaline, the unit spectrum for noradrenaline multiplied by the known noradrenaline concentration was subtracted from each sample spectrum. By doing this, the noradrenaline information in the spectra could be removed and no longer disturb the adrenaline predictions. The same procedure was followed when doing the corrected Unfold PLS-1 regression for noradrenaline. The results from the corrected models is seen in Fig. 2 B, D. All samples are distributed along the $y = x$-line in the corrected plots (B, D), as opposed to the severe scattering of the samples that is seen in the uncorrected plots (A, C). The correlation between the reference values and the cross-validated prediction of the catecholamine concentrations for the corrected spectra were 0.99 and 0.98, and the RMSECVs were 47.2 and 33.2 nmol/L, respectively. For further details on the calibration results see Table 3, where it is obvious that all values were improved substantially when using the corrected spectra for calibrations. These facts show that the Unfold-PLSR models did not differentiate between the two catecholamines. PARAFAC models with one and two components showed the same result as the Unfold-PLSR: only one component was needed to describe this data set adequately, and the emission and excitation loadings were similar for both catecholamine spectra.

This led to the conclusion that, given these instrumental settings, it was not possible to distinguish the fluorescence spectra of adrenaline and noradrenaline. In

order to verify the equivalence of the spectra, the settings of the instrument were changed to provide high resolution. A more detailed description of the use of PARAFAC modelling on the data thus obtained will follow in the next section.

Analysis of set 2

This small data set was, as mentioned above, measured close to the maximum precision allowed by the instrument (see Table 1). Only the results from the measurements with scanning speed of 60 nm/min are presented, because the replicates measured at scan speed 30 nm/min were almost identical and proved exactly the same points. Again, the raw spectra from the fluorometer were transformed using INCA, this time removing only 0th order Rayleigh scatter (bandwidth of 8 nm) as 1st order scatter appeared in a region not measured and also using every second emission wavelength measured. The resulting set had the dimension: 6 samples $\times$ 125 emission wavelengths $\times$ 26 excitation wavelengths, and there were 8.5% missing values. A typical sample is shown in Fig. 3.

Models with two components. As artificial urine solutions were used, it was certain that only two fluorophores, namely adrenaline and noradrenaline, were present. Each would ideally give rise to variation describable by one PARAFAC component. A PARAFAC model with two components was therefore fitted. Each component (ideally holding the characteristics of one fluorophore) consisted of three loading vectors, describing the relative concentration, the estimated emission spectrum and the estimated excitation spectrum, respectively. When models with more than one component are calculated, the loadings for one *mode* (here the modes were concentration, emission and excitation) are joined in a matrix. When plotting the results of a PARAFAC model, one typically plots the loading matrix of each mode separately, as shown in Figs 4, 5 and 6.

If the spectra of adrenaline and noradrenaline are in fact different, one should expect the pure excitation and emission spectra of the two analytes to be resolved by the two-component PARAFAC model. This was, however, not the case, as only the estimated emission and excitation spectra of the first PARAFAC component made immediate sense (Fig. 4), i.e. the first loading of the spectral modes showed smooth peaks and had maxima at the expected wavelengths. The second emission and excitations loadings possibly described the scatter in the

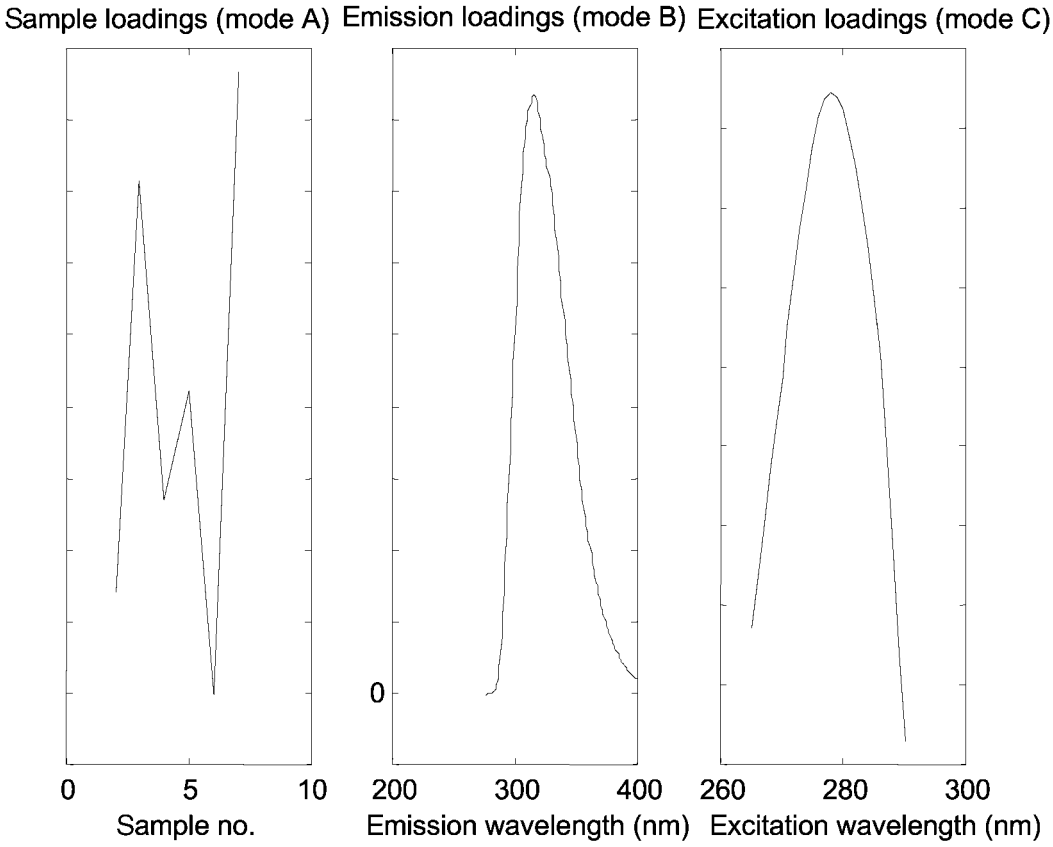

Figure 6. Loadings from a PARAFAC model with one component and no constraints for set 2. The scaling on the ordinate axes is arbitrary.

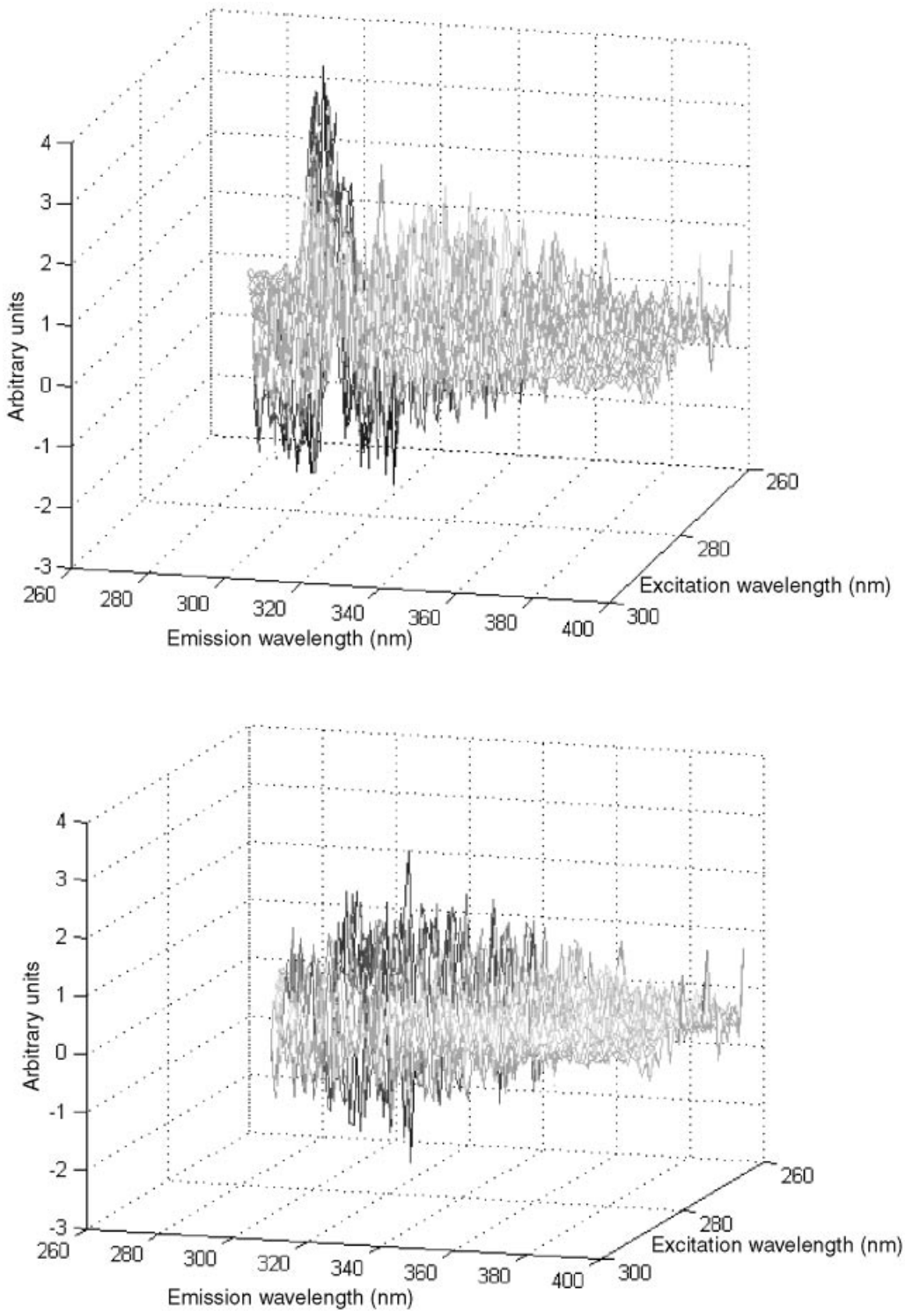

Figure 7. Residuals for one-component PARAFAC model for sample No. 2 in set 2. Top, Raman scatter has not been removed; systematic variation is seen as a line across the landscape. Bottom, Raman scatter has been removed, and no systematic variation is seen.

spectra, but it could also have been pure noise modelling. Further, the second loading in mode A was almost constant and close to zero, which also indicated that no catecholamine information was captured by the second component and that it probably described noise. Constraining the model parameters to non-negativity did not improve the results, and neither did measuring the samples at even lower scan speed (as mentioned, replicates were measured at scanning speed 30 nm/min).

From these results it seems that the emission and

excitation spectra of adrenaline and noradrenaline were identical. To further validate this, the PARAFAC model was fitted, using the additional *a priori* knowledge of the individual concentrations in the samples. Thus, the PARAFAC model was fitted under the constraint that the parameters pertaining to the concentrations (the scalars a_{if} in Eq. 1) were identical to the reference concentrations. In this case, the concentrations were thus fixed *a priori* to be correct and the PARAFAC model only estimated the spectra of the model. Thus, each of the two calculated PARAFAC components were directly forced to find the variation in the excitation and emission modes that reflected the sample concentration variation of the two analytes. This added constraint would make modelling easier and more correct if the data followed the two-component PARAFAC model. The results showed two exactly similar first and second loadings for mode B and C (Fig. 5). The results left only one conclusion possible: it was not possible to separate the catecholamines mathematically using these data. The data were thus considered as reflecting the variation in only one phenomenon, namely the total catecholamine concentration.

Models with one component

Consequently, a PARAFAC model with only one component was fitted to the data. This supported the fact that only one fluorescing compound (or rather two identical ones) as present. Calculation of a PARAFAC model with only one component, where no constraints were used, resulted in perfect catecholamine excitation and emission loadings (Fig. 6).

As mentioned, the same samples were measured with nearly identical instrumental settings on 2 different days. Calculations of PARAFAC models for corrected adrenaline and noradrenaline spectra (same correction procedure as described in the section on *Analysis of set 1*, above) were made separately for the 2 days. Calibrations using the calculated PARAFAC model were then made separately for each day. The calibration results showed quite good accordance with the total catecholamine concentration (Table 2).

Influence of Raman scatter. Calculating the residuals of the one-component model showed the interesting fact that the Raman scatter was not modelled, and could be seen as peaks on a straight line across the residual landscape for each sample (Fig. 7, top). Hence, the PARAFAC modelling was capable of separating the trilinear 'chemical' information from the scatter interference. Subtracting a fluorescence landscape of 'unspiked' artificial urine from the sample data before fitting the PARAFAC model removed the Raman scatter and the residuals showed no systematic variation (Fig. 7, bottom). A calibration using the PARAFAC model, where Raman had been removed, still showed no improvement in the calibration result, and thereby verified that the Raman scatter in this case did not influence modelling significantly.

CONCLUSIONS

The fluorescence landscapes of adrenaline and noradrenaline obtained in artificial urine using an LS50B fluorometer could not be separated using chemometric methods. The spectra were identical when measured with these instrumental settings at ca. pH 3. A new analytical method for adrenaline and noradrenaline using chemometrics and fluorescence will thus have to make use of some way of separating the spectra. In future work it will be investigated whether a derivatizing procedure (e.g. the well-known 3,5,6-trihydroxyindole method described in the Introduction) will be helpful in separating the fluorescence signals of the two compounds.

Acknowledgements

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