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Determination of Catecholamines and Related Compounds by Capillary Electrophoresis with Postcolumn Terbium Complexation and Sensitized Luminescence Detection

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A method has been developed for the determination of catecholamines and related compounds (DA, E, NE, DOPA, DOPAC, DOPAG) in urine samples. The compounds of interest were separated by capillary electrophoresis with a borate buffer as background electrolyte. Detection was based on the sensitized luminescence of terbium ions. Using a homemade postcolumn reactor, a basic solution containing a stoichiometric mixture of terbium(III) chloride and EDTA was added postcolumn to the separation buffer. The ternary catechol—EDTA—terbium complexes formed in the reaction mixture showed a strong luminescence with excitation and emission maxima at 300 and 545 nm, respectively. By optimization of the experimental conditions, zone broadening could be restrained and plate numbers up to 130 000 appeared to be possible. Detection limits found were in the order of $10^{-7}$ mol L$^{-1}$. The catecholic compounds could be determined in urine samples after a standard cleanup and preconcentration procedure.

The measurement of the levels of catecholamines and their metabolites in biological fluids such as urine, plasma, and serum is very important in clinical diagnosis. HPLC coupled with electrochemical detection (ED) or fluorescence detection is most often used for the determination of catecholamines. Compared with ED, fluorescence detection is more robust and therefore presently more widely applied. Catecholamines with their phenolic structure exhibit native fluorescence, with excitation and emission wavelengths around 280 and 310 nm, respectively. However, native fluorescence detection is not often used as an analytical method in HPLC for catecholamines, because of the short Stokes shift and the low sensitivity and selectivity. So far, fluorescence detection of catecholamines and related compounds has been mainly based on pre- or postcolumn derivatization.

Electrochemical and (laser-induced) fluorescence detection methods for catecholamines have also been developed for capillary electrophoresis (CE). Since an electrochemical (amperometric) detector for CE is not yet commercially available, a homemade detection cell has to be used for CE-ED of catecholamines. The detection of catecholamines can also be based on native fluorescence, using a (expensive) deep-UV argon laser or on pre- or postcolumn fluorescence derivatization with naphthalene-2,3-dicarboxaldehyde. Though this latter derivatization method provides high sensitivity for dopamine and norepinephrine, it cannot be used for epinephrine and for metabolites that have no primary amino groups. Because of its miniaturized scale, CE is especially suited for small samples. Most of the CE studies mentioned above were focused on the determination of catecholamines in single cells or cell compartments. However, the (concentration) sensitivity of detection in CE is generally not sufficient for the measurement of catecholamines in body fluids without extensive sample workup.

Lanthanide sensitized luminescence has been applied as an analytical technique for the analysis of organic compounds of biological interest. The advantages of lanthanide sensitized luminescence are a large Stokes shift, narrow emission bands, and long luminescence lifetimes. The energy transfer from the molecule of interest (the donor) to lanthanide ions (the acceptor) normally occurs between the lowest excited triplet state of the donor and the $^5$D state of the lanthanide. The energy transfer can follow an intra- or an intermolecular pathway. The essential requirement for the energy transfer process is that the triplet energy of a donor should be larger than the $^5D$ energy of the acceptor. On the basis of consideration of energy levels and luminescence quantum yields, terbium and europium are preferred for analytical applications. Terbium or europium ions can form stable complexes with various organic ligands and undergo intramolecular energy transfer. This kind of energy transfer is not diffusion controlled because Tb$^{3+}$ or Eu$^{2+}$ ions combine with ligands (donors) by coordinate linkage. Thereby it is a fast process with an energy transfer rate constant that can be as high as $10^{10}$ s$^{-1}$. The quenching of both the triplet energy of the donor

and the luminescence of Tb$^{3+}$ or Eu$^{3+}$ by water or dissolved oxygen in aqueous solution is thereby greatly decreased.

Detection based on terbium or europium sensitized luminescence has been used in HPLC for tetracyclines,17 nucleic acids and nucleotides,18 orotic acid,19 steroids,20 antibiotics,21 theophylline,22 and mycotoxins.23 In HPLC, terbium solution has been introduced into the system mainly in a postcolumn scheme, which is possible because the kinetics of the complexation is fast. In CE, terbium sensitized luminescence has been used for the detection of steroids24 and for orotic acid.25 In these applications, terbium salt had been added to the separation buffer.

The introduction of terbium as a component of the separation buffer in CE resulted in some problems such as multiple component formation, precipitation, and serious adsorption of terbium on the wall of the capillary. Because of the fast kinetics of complexation and low background luminescence of the terbium reagent, it would be attractive to introduce terbium ions in a postcolumn scheme in CE. In previous work, we developed a simple postcolumn reaction system for CE, which has been successfully used for amino acids analysis with OPA as the postcolumn derivatization reagent.26 In the work described in the present paper, we have studied the application of this system for terbium sensitized luminescence detection in CE.

In a number of studies on terbium sensitized (batch) fluorometry, it was mentioned that catechol interferes with the determination of salicylic acid.27,28 In preliminary experiments, we found that catecholamines and some of their metabolites with ortho hydroxy groups can form complexes with terbium in the presence of EDTA, which serves as synergistic ligand and prevents terbium hydrolysis at high pH. When the solutions were excited at the absorption wavelength of catecholamines, the typical terbium emission bands could be observed.

In this paper, a sensitive detection method for catecholamines will be presented, based on a postcolumn reaction scheme for terbium sensitized luminescence in combination with a separation by CE. For detection, a prototype of a new instrument was used with a novel optical design. The method has been applied for the determination of urinary catechols after a simple cleanup procedure.

**EXPERIMENTAL SECTION**

**Apparatus.** A Prince instrument (Prince Technologies, Emmen, The Netherlands) was used for CE. Samples were introduced hydrodynamically (40 mbar, 6 s). Separations were routinely carried out with a 30 kV voltage. The pressure system was made. Fused-silica capillaries, with an internal diameter of 75 µm, were obtained from Composite Metal Services (Hallow, Worcestershire, UK). Pieces of 80 and 12.5 cm (5.5 cm to the window) were used as separation and reaction capillaries, respectively.

For fluorescence detection, a prototype of an Argos 250 instrument (Flux Instruments, Karlsga, Sweden) equipped with a 75 W mercury–xenon lamp, was used. A Schott glass UG11 filter (Varian) and 500 nm cutoff filter (Flux Instruments) were used for excitation and emission, respectively. A HP 3590OC multichannel interface and a Chemstation with HPLC software (Hewlett-Packard, Waldbronn, Germany) were used to collect and process the detector data. Luminescence spectra were recorded with a Waters 474 scanning fluorescence detector (Waters, Milford, MA) and absorption spectra with a PU 8720 UV/visible scanning spectrophotometer (Philips, Eindhoven, The Netherlands). All experiments were carried out under ambient temperature (21 ± 1 °C).

**Chemicals and Solutions.** Dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were obtained from Janssen Chimica, DOPA and epinephrine (E) from Sigma Chemical Co., norepinephrine (NE) from Fluka-BioChemika, and 3,4-dihydroxyphenyl glycol (DOPAG) from Aldrich. Terbium chloride (TbCl$_3$$ \cdot $6H$_2$O) was obtained from Acros Organics. All other reagents used, obtained from standard suppliers, were of analytical grade purity. Solutions were prepared with subboiled, deionized water. Stock solutions of catecholamines were prepared in 0.1 mol L$^{-1}$ hydrochloric acid and contained 0.4 mmol L$^{-1}$ sodium sulfite to prevent oxidation; the solutions were stored in a refrigerator. Working solutions of catecholamines were prepared fresh daily by dilution of the stock solutions. All solutions were deaerated with helium before use.

**Urine Sample Cleanup Procedure.** Fresh urine samples were adjusted to pH 3 with 6 mol L$^{-1}$ HCl solution and stored in a refrigerator if not immediately used. To 10 mL of sample, 1 mL of 0.2 mol L$^{-1}$ EDTA and 0.1 mL of 0.5 mol L$^{-1}$ ascorbic acid were added. Before cleanup, the pH of the solution was adjusted to 8.5 with NaOH solution. The sample was then loaded onto a light alumina B cartridge (Waters), which had been prewetted with water. The cartridge was washed by two volumes of 2 mL of water. The catecholamines were eluted with 1 mL of 0.1 mol L$^{-1}$ HCl. The eluate was directly injected onto the capillary.

**RESULTS AND DISCUSSION**

**Absorption and Luminescence Characteristics.** The absorption spectra of catecholamines in 1 mmol L$^{-1}$ HCl display absorbance maxima at 221 and 280 nm. The absorbance wavelengths are red shifted to 238 and 300 nm in the presence of Tb$^{3+}$ and EDTA in a solution of pH 11. This wavelength shifts indicate the formation of terbium–catecholamines–EDTA ternary complexes. Normally, catecholamines are not stable and are quickly oxidized in basic environment. However, when complexed with terbium and EDTA, catecholamines were found to be stable even in 0.1 mol L$^{-1}$ NaOH solution, as could be observed from the color

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of the solutions. Without terbium and EDTA, catecholamine solutions in 0.1 mol L\(^{-1}\) NaOH change quickly from colorless to red and then to yellow. With terbium and EDTA, the solutions stay colorless. The stability of the substances in basic aqueous solution is increased because the ortho hydroxy groups are coordinated with terbium.

The luminescence of terbium salts in aqueous solution is very weak due to the weak absorption of the metal ion itself. The molar absorptivity\(^{29}\) corresponding to the \(^3D_{4}\) level of the terbium ion (at 20 500 cm\(^{-1}\)) is 0.029 mol\(^{-1}\) cm\(^{-1}\). When an organic ligand having a strong absorption in the UV range is present, terbium can accept the energy from the lowest triplet energy of the ligand (donor). As a consequence, the luminescence of terbium can be dramatically increased. When the ligand molecule is excited, the excited singlet state has three deactivation pathways, i.e., radiative transition accompanied with fluorescence emission (\(S_1 \rightarrow S_0\)), nonradiative transition to the triplet state (\(S_1 \rightarrow T_1\)) by intersystem crossing (ISC), and nonradiative deactivation to the ground state (\(S_1 \rightarrow S_0\)). The excited triplet state has three deactivation pathways too: radiative deactivation accompanied with phosphorescence (\(T_1 \rightarrow S_0\)), nonradiative transition to the excitation state of terbium through energy transfer (ET), and nonradiative deactivation to the ground state of the ligand (\(T_1 \rightarrow S_0\)). The efficiency of the intramolecular energy transfer is dependent on several factors. First, the lowest excitation triplet energy of the donor should be higher than the \(^3D_{4}\) energy level of terbium. Next, the energy transfer rate should be fast enough to compete with the nonradiative deactivation of the triplet energy. Finally, the rate and probability of the intersystem transition of the donor from excited singlet state to triplet state should be high.

The phosphorescence emission wavelengths of catecholamines (measured at 77 K) are between 409 and 417 nm (around 23 800–24 000 cm\(^{-1}\)).\(^{30}\) This means that the \(T_1\) energy of catecholamines is 3800–4000 cm\(^{-1}\) higher than the energy of \(^3D_{4}\) state of Tb\(^{3+}\). At room temperature, phosphorescence is hardly observed in aqueous solutions since nonradiative deactivation is much faster than radiative deactivation. However, when the energy transfer rate can compete with the nonradiative deactivation rate, energy transfer from catecholamines to terbium can occur. The luminescence spectrum of the terbium–epinephrine–EDTA complex is shown in Figure 1 (curve a). The wavelength of maximum excitation for the complex in CAPS buffer at pH 11 is at 300 nm. The typical terbium luminescence at this excitation wavelength can be observed, with emission at 490 \(\lambda\) \(^{3}D_{4} \rightarrow \gamma F_{2}\)\), 545 \(\lambda\) \(^{3}D_{4} \rightarrow \gamma F_{3}\)\), and 595 nm \(\lambda\) \(^{3}D_{4} \rightarrow \gamma F_{4}\). The spectra do not show any emission in the range from 300 to 450 nm, corresponding to the native fluorescence or phosphorescence wavelengths of catecholamines. When excited at 280 nm, the native fluorescence excitation wavelength of the catecholamines, the intensities of terbium luminescence were decreased but native fluorescence or phosphorescence still could not be observed.

The fluorescence spectrum of the complex in the presence of cesium chloride is also shown in Figure 1 (curve b). The luminescence intensity is increased because the heavy atom effect of cesium. From the energy transfer scheme it can be understood that the intensity of terbium luminescence depends on the ISC probability. It is well-known that the presence of heavy atoms can increase the spin–orbit coupling effect and thereby the ISC probability. We examined the effect of various heavy-atom ions (I\(^{-}\), Ba\(^{2+}\), Cs\(^{+}\), Rb\(^{+}\) on the luminescence intensity. Among them, cesium was found to be the most effective. The luminescence for epinephrine was increased by a factor of 3.

In Figure 2, the fluorescence intensities of catecholamines in different solutions are compared. In all (flow-through) measurements, a UG11 filter was used for excitation and a cut-off filter (>470 nm) for emission. Under these conditions, native fluorescence of catecholamines was not observed in aqueous solution at low pH. In basic solutions, especially epinephrine can show a strong luminescence at longer wavelengths, which has been attributed to the phosphorescence of an oxidation product of E.\(^{30}\) After preparation of the solution, it took some time to reach the maximum luminescence intensity (1–3 min) and the signal decayed soon after reaching the maximum. The stability and reproducibility of the signal were too poor for this reaction to be used for analytical purposes. Moreover, not all catecholamines

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capillary, the applied pressure was 30 mbar, giving a reaction time controlled by the pressure; routinely, with a 75 µm i.d. reaction capillary, the applied pressure was 30 mbar, giving a reaction time of 10 s. The high voltage used for electrophoresis was grounded by a platinum electrode fixed in the reagent reservoir.

For fluorescence detection, (a prototype of) an instrument with a novel optical principle was used. The light from a mercury or mercury–xenon lamp is guided, after filtering, by an optical fiber to the detection unit. The excitation light is directed on the inner diameter of the capillary, perpendicular to the capillary axis. Of the light emitted by fluorescent compounds in the solution, a part (~15% to either side) is trapped inside the capillary by total reflection at the silica–air interface. At some distance (~1 cm) from the site of excitation, the trapped light is decoupled from the capillary by means of an optically transparent (plexiglass) cone around the capillary. Optical contact is made with glycerine. The cone reflects the emission light in a direction approximately parallel to the capillary axis, toward the tip of a second optical fiber, leading to a second filter and a photomultiplier tube. The optical principle is illustrated by Figure 4. The advantage of this principle is that reflected or scattered excitation light, which will have a direction perpendicular to the capillary axis, is almost completely kept away from the detector. In preliminary experiments with OPA derivatives of amino acids, we found an improvement of the signal-to-noise ratios by a factor of 50–100, compared to a previously used modified HPLC detector.

Optimization of the Postcolumn Reagent. For the optimization of the reagent composition, epinephrine was used as the model compound. In Figure 5a, the influence of the solution pH on the luminescence intensity is shown. The observed pH dependency suggests that ionization of the hydroxy groups of the catechols is necessary for the formation of ternary complexes. Another explanation could be that generally the phosphorescence of phenolic derivatives is increased while the fluorescence is decreased at high pH. This means that the probability of intersystem crossing, and with that of energy transfer, is increased. A maximum luminescence intensity was observed from pH 11 to 13. In further experiments, CAPS buffer with a pH of 11 was used, because at higher pH the risk of terbium hydrolysis and precipitation is increased.

The influence of the terbium chloride concentration in the reagent on the luminescence intensity is shown in Figure 5b. The experiments showed that the luminescence intensity was maximum when the terbium concentration exceeded 0.75 mmol L⁻¹. Apparently, the complex formation is virtually complete at these concentrations. In following experiments, 2 mmol L⁻¹ of Tb³⁺ was used in the reagent.

EDTA plays an important role. At high pH, EDTA prevents terbium hydrolysis and precipitation. When the ratio of EDTA to Tb was lower than stoichiometric, white precipitate was gradually produced, which lead to serious blockage of the capillary. We have tried to use 50 mmol L⁻¹ NaOH as a postcolumn reagent in CE, with a reaction time of ~10 s. However, only a very small peak for NE could be observed.

When a ternary complex was formed with terbium and EDTA, a strong luminescence corresponding to the emission of terbium(III) was produced with all catecholic compounds studied, which was further increased in the presence of Cs⁺. Compared with the fluorescence intensity in water or in a 50 mmol L⁻¹ NaOH solution, an enhancement of 30 times was obtained for DA.

Instrumentation. The postcolumn reaction system used was a modification of the one that has been described in detail before. Figure 3 shows it schematically. A homemade Kel-F reagent reservoir that can contain 1–2 mL of solution was used. The fused-silica separation and detection capillaries were connected with each other by means of a porous PTFE tube (2 mm o.d., 0.35 mm i.d.), leaving only a very narrow gap between them. The porous tube fitted as a tight sleeve over the two capillaries, so that they were firmly held in place. After installation of the porous tube, the capillaries were fixed with finger-tight fittings in the reservoir. The reagent solution was introduced through the porous tube into the reaction capillary by means of (air) pressure, which could be provided and controlled by the injection system of the CE instrument. During electrophoresis, the same pressure was applied at the reactor and the inlet buffer vial, to prevent distortion of the flat electroosmotic flow profile in the separation capillary by pressure-induced flow. The reaction time can be controlled by the pressure; routinely, with a 75 µm i.d. reaction capillary, the applied pressure was 30 mbar, giving a reaction time of 10 s. The high voltage used for electrophoresis was grounded by a platinum electrode fixed in the reagent reservoir.

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and the porous tube. With an increase of the EDTA to Tb³⁺ ratio above 1, the luminescence intensity of epinephrine is decreased (Figure 5c). This behavior suggests that the excess EDTA molecules coordinate with terbium, so that the opportunity for catecholamines to form ternary complexes is decreased. Moreover, the reagent background fluorescence was increased (dashed line in Figure 5c).

In batch experiments, cesium ions were found to be the most effective for the luminescence enhancement by the heavy-atom effect. The influence of cesium chloride concentration in the reagent is shown in Figure 5d. A maximum is reached above a concentration of CsCl of 0.5 mol L⁻¹. A concentration of 0.8 mol L⁻¹ was used in further experiments.

Separation of Catecholamines. Although a CE separation of catecholamines at low pH has been reported in the literature,² we did not succeed in obtaining a complete separation of DA, E, and NE in acidic buffers. In neutral solution (Tris buffer at pH 7.5), when the catecholamines are positively charged, the separation required the addition of sodium dodecyl sulfate (SDS). However, the peaks obtained were very broad and the resolution was poor. According to their pKa values, the catecholamines will carry a negative charge at a pH higher than 9. However, catecholamines are easily oxidized at higher pH. To prevent oxidation, EDTA (0.5 mmol L⁻¹) and ascorbic acid (1 mmol L⁻¹) were added to the buffer, while the buffer was prepared daily and degassed by helium flushing. In CAPS buffer at pH 10, the separation of DA and E was still quite poor and the peaks were broad. Even at this high pH, the amines showed a strong interaction with the wall of the capillary. Peak shapes of the catecholamines could be improved to a certain extent by adding 2 mmol L⁻¹ triethylamine (TEA) to the buffer. All catechols could be well separated in boric acid buffer at pH 10. The improvement of the separation in boric acid buffer is most probably due to the complex reaction between borate ions and the orthohydroxy group of the catecholamines. Figure 6 shows the electropherogram obtained with a standard solution of six catecholic compounds in a 30 mmol L⁻¹ boric acid buffer as the background electrolyte.

With a 75 μm i.d. detection capillary, plate numbers for the peaks ranged from typically 50 000 for the amines to 130 000 for the acidic compounds. We have also used a 50 μm reaction capillary. Experimental conditions were kept the same except for the pressure applied to the reaction vessel. This pressure was increased to 150 mbar to keep the reaction time at ∼10 s. Higher pressure applied with the 50 and 75 μm capillaries to obtain a 10 s reaction time was 150 and 30 mbar, respectively. For other experimental conditions, see text.² By comparison with the luminescence intensity of DA in a 50 μm i.d. capillary, the relative standard deviations of the retention times, peak heights, and peak areas for the acidic compounds (DOPA, DOPAG, DOPAC), were around 0.6, 2, and 4%, respectively; for the catecholamines around 0.9, 6, and 8% respectively (n = 5). The lower reproducibility for the amines may be caused by the lower stability of these compounds in high-pH solution.

Analysis of Urine Samples. The method was used for the analysis of urine samples. In a urine sample, there are many

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**Figure 5.** Influence of the reagent composition on the luminescence intensity of epinephrine. (a) Influence of the pH, with 2 mmol L⁻¹ Tb³⁺ and EDTA. (b) Influence of the terbium concentration with equivalent concentration of EDTA. (c) Influence of the EDTA concentration, with 1 mmol L⁻¹ Tb³⁺; on the signal for E (solid line) and the background fluorescence intensity (dotted line). (d) Influence of the concentration of CsCl.

**Figure 6.** Separation of catecholic compounds in 30 mmol L⁻¹ boric acid buffer at pH 10. Concentration of catecholamines 1 × 10⁻⁶ mol L⁻¹ each. For conditions, see text. Peaks: (1) DA; (2) E; (3) NE; (4) DOPAG; (5) DOPA; (6) DOPAC.

**Table 1.** Plate Numbers (N) and Relative Luminescence Intensities (r) with 50 and 75 μm i.d. Reaction Capillaries

<table>
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<th>compd</th>
<th>50 μm N</th>
<th>50 μm r</th>
<th>75 μm N</th>
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<td>94 000</td>
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² The pressure applied with the 50 and 75 μm capillaries to obtain a 10 s reaction time was 150 and 30 mbar, respectively. For other experimental conditions, see text.
When the terbium postcolumn complexing reagent was used, only DA and DOPAC, which are normally present in higher concentrations, could be identified as new peaks (Figure 7b), while signals for other catechol compounds were not discernable. Therefore, a sample cleanup and preconcentration procedure was applied, using a light alumina B cartridge. Samples collected from a healthy individual were treated according to the procedure described in the Experimental Section. With this procedure, 10 mL of the sample are finally collected in 1 mL of eluate that can be injected directly on the capillary. Recoveries calculated by comparing peak areas from experiments with standard solutions and spiked urine samples are given in Table 3. Clearly, the column and/or the procedure are not yet optimal for the preconcentration of the acidic compounds.

CONCLUSIONS
It has been shown that the method studied provides the sensitivity and the selectivity required for the determination of catecholamines in urine samples. The obtained sensitivity is mainly due to the novel optical design of the detector used, which is much better than that of other lamp-based fluorescence detectors we have previously used in CE. In terms of sensitivity, the terbium sensitized luminescence scheme is not even very favorable; with OPA derivatives of amino compounds, 5–10-fold lower detection limits could be obtained. On the other hand, the terbium method gives a useful selectivity for the catecholic group of the compounds under study. This allowed the use of a standard preconcentration method for the urine samples without serious background interference.

Postcolumn addition of terbium ions may also be of value for the detection of compounds with a catecholic group after separa-

<table>
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<th>compd</th>
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<th>fmol</th>
<th>compd</th>
<th>LOD&lt;sup&gt;a&lt;/sup&gt; 10&lt;sup&gt;-7&lt;/sup&gt; mol L&lt;sup&gt;-1&lt;/sup&gt;</th>
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<td>DOPA</td>
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<td>DOPAC</td>
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<sup>a</sup> S/N = 2.

<table>
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<th>recovery (%) mean ± std (n=4)</th>
<th>urinary concn (µmol L&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<tr>
<td>DA</td>
<td>70 ± 3</td>
<td>1.14</td>
</tr>
<tr>
<td>E</td>
<td>85 ± 6</td>
<td>0.01</td>
</tr>
<tr>
<td>NE</td>
<td>83 ± 4</td>
<td>0.06</td>
</tr>
<tr>
<td>DOPAG</td>
<td>65 ± 14</td>
<td>0.20</td>
</tr>
<tr>
<td>DOPA</td>
<td>65 ± 9</td>
<td>0.05</td>
</tr>
<tr>
<td>DOPAC</td>
<td>51 ± 14</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Figure 7. Electropherograms of a 1:1 diluted urine sample, with water (a) or with terbium reagent (b) added postcolumn. Numbers of identified peaks as in Figure 6.

Figure 8. Electropherogram of a urine sample after cleanup and preconcentration on an alumina B precolumn, with terbium reagent added postcolumn. For conditions, see text. Peak numbers as in Figure 6.

Figure 8. Electropherogram of a urine sample after cleanup and preconcentration on an alumina B precolumn, with terbium reagent added postcolumn. For conditions, see text. Peak numbers as in Figure 6.
tion by HPLC. One of the inherent advantages of the terbium system, the long luminescence lifetime, has not yet been exploited. The application of time-resolved luminescence detection might result in a further improvement of the sensitivity and selectivity. Work in this direction is now in progress.

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