# Capillary Electrophoresis Method for the Determination of Amino Acids in Pharmaceutical Samples Based on Precolumn Derivatization Using 1,2-Naphthoquinone-4-Sulfonate

**Rosa M. Latorre, Javier Saurina,** and **Santiago Hernández-Cassou***
Department of Analytical Chemistry, University of Barcelona, Diagonal 647, 08028, Barcelona, Spain

## Abstract

**A new method for the determination of amino acids by capillary electrophoresis based on derivatization with 1,2-naphthoquinone-4-sulfonate is developed. The separation of amino acid derivatives can be performed in a fused-silica capillary with a 40mM aqueous sodium tetraborate solution–isopropanol background electrolyte solution (3:1, v/v) under an applied potential of 30 kV. Glutamic and aspartic acids and cysteine can be separated faster in a 20mM sodium tetraborate solution at 15 kV. Amino acid derivatives are spectrophotometrically detected at 480 nm. The precapillary derivatization is developed for 5 min at 65°C and pH 10. The linear range for lysine is up to $1.25 \times 10^{-3}$ M, the repeatability is 3.0%, and the detection limit is 0.67 pmol when injecting 22.5 nL. The method is applied to the determination of amino acids in pharmaceutical samples. Good agreement is found between the method proposed and the standard method.**

## Introduction

The determination of amino acids is generally performed using high-performance liquid chromatography (HPLC) with spectroscopic detection. Because the direct detection of amino acids provides low sensitivity, in most cases, a chemical deriva-tization of amino acids is required in order to increase the response. For this purpose, both pre- and postchromatographic derivatization methods have been proposed (1). Reagents that carry out amino acid derivatizations are mainly based on the reactivity of the amino group. Features, advantages, and disadvantages of both techniques have been widely discussed elsewhere.

In recent years, capillary electrophoresis (CE) has gained popularity as a separation technique for routine analysis, and it has widespread applications in many fields of analytical chemistry (2). In this way, CE is being introduced as a suitable tool for the determination of amino acids (3). The advantages of CE over HPLC are the smaller volumes of samples, reagents, and buffers required and the higher resolution of the electrophoretic separations. Additionally, the time of analysis is, in general, shorter than that needed in HPLC. In contrast, the main drawback of CE is its lower sensitivity in comparison with HPLC.

Reagents applied to the precolumn derivatization of amino acids in HPLC are, in general, suitable for precapillary derivatization in electrophoresis. Dansyl chloride was originally introduced by Jorgenson and Lukacs (4) for the fluorogenic labelling of primary and secondary amino acids in CE; the resolution can be improved by micellar electrokinetic chromatography (MEKC) (5). *O*-phthaldialdehyde (OPA) reacts with primary amino acids in the presence of a reducing agent, producing fluorogenic isoindole derivatives (6). Its interest in precapillary labelling of amino acids is rather limited, owing to the poor stability of the derivatives. Nevertheless, this stability can be enhanced by using thiol compounds such as ethanothiol (7). The postcolumn reaction with OPA in CE using a porous tube to supply the reagent has also been described (8). Naphthalene dicarboxaldehyde (NAD) was proposed as an alternative to OPA for the formation of stable isoindole derivatives in the presence of cyanide (9). NAD derivatives can be separated by CZE or MEKC (10). Phenylisothiocyanate (PITC) has been used in the precapillary derivatization of amino acids to form phenylthiohydantoinic compounds that can be detected by spectrophotometry or fluorometry. The separation of PITC derivatives has been accomplished by CZE (11) and MECK (12). The sensitivity of the detection can be enhanced by using fluorescein isothiocyanate, which yields highly fluorescent derivatives (13). 9-Fluorenylmethyl chloroformate (14), 2-(9-anthryl) ethyl chloroformate (15), 3-(4-carboxybenzoyl)-2-quinolinecarboxyaldehyde (16), and 1-methoxycarbonylindolizine-3,5-decarbaldehyde (17) have also been used for amino acid analysis by CE with spectrophotometric or fluorometric detection.

In this paper, a new method based on the precapillary derivati-

* Author to whom correspondence should be addressed.

zation of amino acids with 1,2-naphthoquinone-4-sulfonate (NQS) followed by the electrophoretic separation of the corresponding derivatives is developed. The derivatization reaction is performed in batch for 5 min at 65°C and pH 10. Conditions for the quantitative formation of the amino acid derivatives were previously investigated (18). The separation of amino acid derivatives by CE is carried out at 30 kV in a fused-silica capillary by using a 40mM sodium tetraborate–isopropanol (3:1, v/v) solution as a background electrolyte.

Some relevant features of NQS are its free water solubility, mild reaction conditions for primary and secondary amino acids, and low cost. In addition, NQS derivatives can be detected in the visible range; in CE, this may result advantageously with respect to the laser-induced fluorescence detection in terms of versatility, availability of the instrument, and cost. In comparison with other spectrophotometric reagents whose derivatives usually absorb in the ultraviolet (UV) range, this method improves the selectivity of the detection, avoiding electropherograms with additional peaks from absorbing compounds present in the sample matrix that potentially interfere. NQS has been applied successfully to the analysis of amino acids using flow-injection (19), continuous-flow (20), stopped-flow (21), and liquid chromatography methods (18,22).

## Experimental

### Reagents and solutions

All solutions were prepared with Milli-Q (Millipore, Milford, MA) water.

The background electrolyte solution for the separation of common amino acid derivatives was composed of 40mM sodium tetraborate and isopropanol (3:1, v/v). This solution was prepared from boric acid–sodium hydroxide (analytical reagents, Merck, Darmstadt, Germany) and isopropanol (HPLC grade, Romil, Cambridge, U.K.). An alternative background electrolyte solution for the separation of derivatives of acidic amino acids and cysteine was a 20mM sodium tetraborate solution. Acetone (HPLC grade, Carlo Erba, Milano, Italy) was used as a marker of the electroosmotic flow. Sodium dodecylsulfate and sodium octylsulfate (analytical grade, Merck) were the surfactants for MEKC studies.

NQS (analytical grade, Carlo Erba) and a 37% (w/w) hydrochloric acid solution (analytical grade, Merck) were used to prepare the reagent solution consisting of 0.03M NQS in 0.1M HCl. The buffer solution for the development of the reaction was 0.05M sodium borate–0.09M sodium hydroxide (analytical reagent, Merck). A 0.016M sodium dihydrogen citrate–0.038M sodium hydrogen citrate solution (analytical reagent, Merck) was used to stabilize the amino acid derivatives after reaction.

Amino acids were purchased from Merck (all analytical grade): lysine (Lys), tryptophan (Trp), leucine (Leu), tyrosine (Tyr), valine (Val), hyroxyproline (Hyp), alanine (Ala), threonine (Thr), glycine (Gly), glutamic acid (Glu), cysteine (Cys), ornithine (Orn), phenylalanine (Phe), isoleucine (Ile), aspartic acid (Asp), arginine(Arg), proline (Pro), methionine (Met), asparragine (Asp), serine (Ser).

### Apparatus

A Beckman (Fullerton, CA) P/ACE capillary electropherograph with a diode array spectrophotometer connected on-column was used. Multichannel monitoring of the amino acid derivatives was performed at 305, 360, and 480 nm. Electropherograms were processed with a compatible computer using the Beckman P/ACE Station version 1.0 software. Some preliminary studies were carried out with a Kontron (Madrid, Spain) Eureka 2000 capillary electropherograph. Applied potentials were in the range of 10 to 30 kV. Fused-silica capillaries (supplied by Tecknokroma, Barcelona, Spain) of 75-µm i.d. (375-µm o.d.) with an effective length of 58 cm and total length of 70 cm were used. The pH was adjusted using a Crison (Barcelona, Spain) 517 pH meter with a combined glass electrode Orion (Espoo, Finland) 9102 SC. A Pharmacia LKB (Cambridge, U.K.) amino acid analyzer was used for the determination of amino acids with the standard method.

### Sample treatment

The pharmaceutical samples listed below were analyzed in order to validate the accuracy of the proposed method for the determination of amino acids: Salvacolon (oral suspension from Laboratorios Salvat, Esplugues de Llobregat, Spain), Troforex pépsico (oral suspension from Laboratorios Reig Jofré, Barcelona, Spain), Trimetabol (oral solution from J. Uriach, Barcelona, Spain), Pranzo (oral solution from Laboratorios Viñas, Barcelona, Spain), Policolinosil (tablets from Laboratorios Medea, Barcelona, Spain), and Tebetane (capsules from Laboratorios Elfar-Drag, Fuenlabrada-Madrid, Spain).

Sample solutions to be used for the NQS derivatization were prepared as follows:

Pranzo, Troforex pépsico, Trimetabol, and Salvacolon were diluted 10-, 200-, 500-, and 500-fold, respectively, with water.

For the Policolinosil sample, 0.75 g of powdered product was dissolved in 100 mL of water. The solution obtained was filtered through a nylon membrane (Schleicher & Schuell, Northeim, Germany) with a 0.45-µm pore size.

Tebetane powder (0.30 g) was dissolved in 100 mL of water and filtered through a nylon membrane with a 0.45-µm pore size. The resulting solution was diluted 10-fold with water.

### Precapillary derivatization

Amino acid derivative solutions were obtained by mixing 250 µL of sample solution with 250 µL of NQS solution and 250 µL of borate buffer solution. The reaction was developed in a water bath at 65°C for 5 min (pH of the reacting mixture was 10.0). Subsequently, 250 µL of citrate solution was added to acidify the mixture in order to stop the degradation of excess reagent and stabilize the derivatives. The resulting solution was filtered through a nylon membrane (0.45-µL pore size) and injected into the electrophoretic system.

### Capillary electrophoresis conditions

Sample solutions were injected by pressure at 0.5 psi for 5 s. Two different separation conditions were established. The separation of amino acid derivatives was performed under an applied potential of 30 kV using a 40mM sodium tetraborate aqueous solution (pH 9.2) and isopropanol (3:1, v/v) as a background elec-

trolyte. Acidic amino acids and cysteine were separated faster at 15 kV with a 20mM sodium tetraborate electrolyte solution. In both cases, the capillaries were thermostatted at 25°C.

All new capillaries were flushed with 0.1M NaOH solution for 20 min. The capillaries were then rinsed with water for 5 min and conditioned with background electrolyte solution. In series of the analyses, the capillary was washed with water for 3 min and equilibrated with the background electrolyte solution for 5 min.

### Standard method for the determination of amino acids

The amino acid autoanalyzer based on the ninhydrin method (23) was used for the determination of amino acids in pharmaceutical samples. The amino acids were separated by cation exchange in an Ultropack column (200 × 4-mm i.d., 8-µm particle size) using lithium solutions. An elution gradient based on increasing pH and ionic strength was used. The postcolumn derivatization of amino acids was performed online by coupling the outlet of the analytical column to a ninhydrin channel. This derivatization was developed at 135°C in a PTFE coil with a 0.3-mm i.d. Derivatives were spectrophotometrically detected at 570 and 440 nm in a 15-mm path-length flow cell and 8 µL of dead volume.

## Results and Discussion

The reaction between amino acids and NQS takes place through the amino group to yield (at first) the corresponding alquilamino derivatives, which are detectable spectrophotometrically. However, when the mixture of amino acid and NQS is allowed to react for a longer time, some side products may appear. Among them, a high-absorbing dark-violet compound that, in the electropherograms, is referred to as "DC" is especially relevant (24). Also, NQS can undergo degradation processes such as hydrolysis and oxidation in the mixture medium. As a result, additional peaks (indicated in the figures with an asterisk) corresponding to unreacted NQS species and degradation compounds appear in the electropherograms.

The optimization of the reaction conditions was carried out in order to provide a general quantitative procedure for the derivatization of amino acid mixtures. The behavior of each amino acid was different, because some of them (such as Pro, Trp, or Lys) could be derivatized quicker and in milder conditions than others (Glu and Asp) that required more drastic derivatization conditions. On the other hand, amino acid derivatives might decompose and lead to side products when temperature and reaction time increase (22). In this way, the derivatization conditions proposed in this study were a suitable compromise to attain a high reaction yield (near 100%) for all amino acids and, at the same time, minimize the degradation of derivatives.

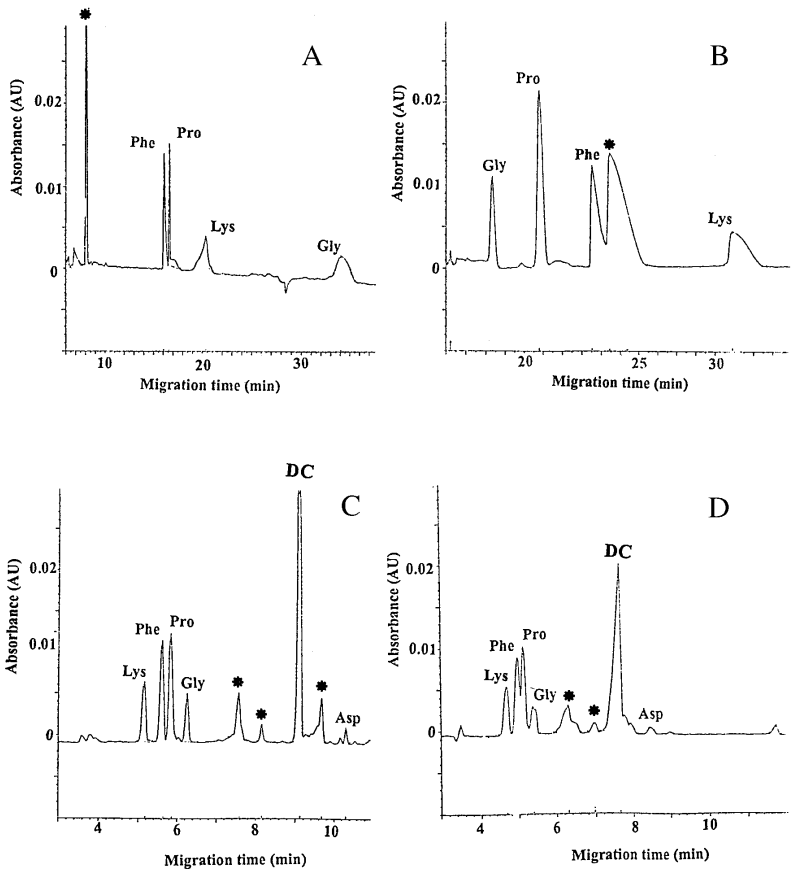


**Figure 1.** Electropherograms of amino acid derivatives using different background electrolyte solutions (at different pH values). Background electrolyte solutions: 20mM phosphoric acid–10mM hydrochloric acid (pH 2.1) (A), 10mM sodium dihydrogen citrate–10mM sodium monohydrogen citrate (pH 4.7) (B), 20mM sodium tetraborate (pH 9.2) (C), 10mM sodium monohydrogen phosphate–10mM sodium phosphate (pH 12.3) (D). DC indicates degradation compound. An asterisk (*) indicates an NQS species. Electrophoretic conditions: potential, 25 kV; injection time, 5 s; temperature, 25°C. Reaction conditions: reaction time, 5 min; reaction temperature, 65°C; reagent solution, $3 \times 10^{-2}$ M NQS and 0.1M HCl; buffer solution pH, 10; amino acid solution, $2 \times 10^{-3}$ M each; volume of reagent, buffer, and amino acid solution, 250 µL each.

The derivatization conditions are mild enough to avoid the break down of Arg to Orn, Asn to Asp, and Gln to Glu. Amino acids with two amino groups (such as Lys or Orn) could give mono- or di- derivatives; however, the NQS concentration is sufficiently high to form the di- derivatives only, avoiding undesired mixtures that may hinder the quantitation. No evidence of side reactions through the sulfidril group of Cys was found, and then, only a derivative was obtained under the reaction conditions.

The resulting derivatives retain the carboxylic group from the amino acid; hence, a negatively charged species from the deprotonation of these groups can be formed. Preliminary studies were addressed to examine the migration of the NQS derivatives in a potential field. As a result of the electroosmotic flow to the cathode in fused-silica capillaries, an apparent migration of amino acid derivatives toward the cathode was observed, with the highest apparent velocity for NQS derivatives of basic amino acids such as Arg, Lys, or Orn derivatives.

### Study of separation conditions

*Effect of pH*

The effect of pH on the separation was studied in the range of 2.1 to 12.3 using various citrate, phosphate, and borate buffer solutions as background electrolytes (Figure 1). At pH 2.1, the electroosmotic flow was very low; thus, only the peaks of basic amino acids appeared at reasonable migration times. At pH 4.7, the peaks of neutral amino acids were detected, but the shapes of some of them presented wide tails. In basic medium, even Asp and Glu derivatives moved to the cathode quite rapidly, and no peak tailing was observed. From this study, a borate buffer was chosen for further experiments, because this also permitted the rapid equilibration of the capillary.

Various 20mM tetraborate background electrolyte solutions were prepared by adding NaOH or HCl in the pH range of 8.6 to 9.8. For most amino acid derivatives, migration times were not affected, because the electroosmotic flow was very rapid (and almost constant) in all cases. On the other hand, pH influenced the peak shape and the resolution, which was optimum at pH 9.2. The pH of the buffer borate solution was finally chosen as 9.2.

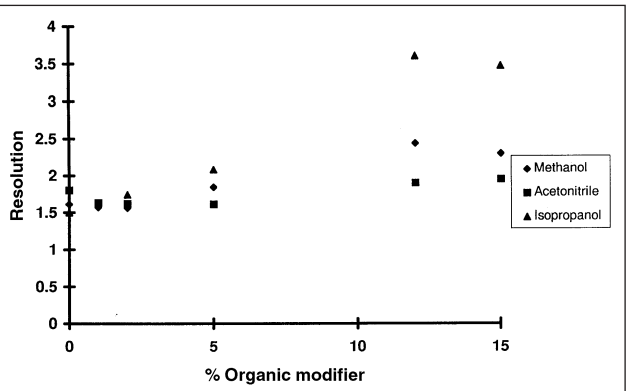


**Figure 2.** Effect of the addition of organic modifiers on the resolution of 2 close peaks (Phe and Gly derivatives). Electrophoretic conditions: potential, 25 kV; injection time, 5 s; temperature, 25°C; background electrolyte solution, 20mM sodium tetraborate (pH 9.2) and solvent. Reaction conditions: reaction time, 5 min; reaction temperature, 65°C; reagent solution, $3 \times 10^{-2}$ M NQS–0.1M HCl; buffer solution pH, 10; amino acid solution, $5 \times 10^{-4}$ M each; volume of reagent, buffer, and amino acid solution, 250 µL each.

*Effect of ionic strength*

The effect of ionic strength on the separation of amino acid derivatives was studied by preparing tetraborate solutions in the concentration range of 10 to 55mM. The results show a continuous increase in the migration time with the ionic strength. At the same time, the resolution was also improved by increasing this variable. However, owing to the progressive increase in the current intensity, a limitation in the buffer concentration at 40mM sodium tetraborate is recommended.

*Choice of modifiers*

The effect of the addition of organic modifiers to the background electrolyte solution on the migration time of the amino acid derivatives was studied. The organic solvents considered were methanol, acetonitrile, and isopropanol. Figure 2 shows the effect of each solvent on the resolution of 2 close peaks. Results indicated that the best separation was achieved with isopropanol, and consequently, this solvent was selected as the modifier of the sodium tetraborate electrolyte solution.

Subsequent studies were aimed at the optimization of the isopropanol concentration in the electrolyte solution. The percentage of solvent was varied in the range of 0 to 35% (v/v). The results showed that the migration times of amino acid derivatives increased with increasing isopropanol percentage. An electrolyte solution of 40mM sodium tetraborate–isopropanol (3:1, v/v) was used for the resolution of derivatives from neutral and basic amino acids, whereas the separation of derivatives from acidic amino acids could be more quickly accomplished without isopropanol.

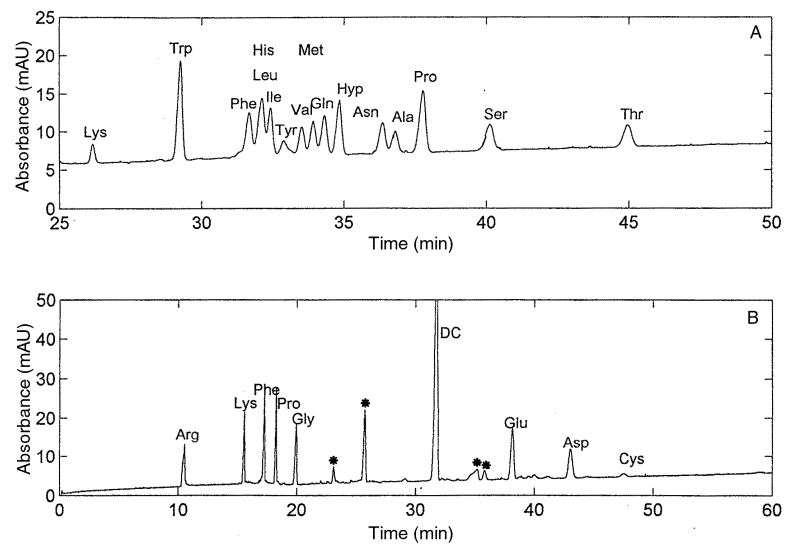


**Figure 3.** Electropherograms obtained for the separation of amino acid derivatives of a standard solution: hydroorganic buffer (A) and aqueous buffer (B).
Hydroorganic buffer: 40mM sodium tetraborate (pH 9.2)–25% isopropanol (v/v). Electrophoretic conditions: potential, 30 kV; injection time, 5 s; temperature, 25°C. Aqueous buffer: 20mM sodium tetraborate (pH 9.2). Electrophoretic conditions: potential, 15 kV; injection time, 5 s; temperature, 25°C. Reaction conditions were the same as in Figure 2. DC indicates a degradation compound. An asterisk (*) indicates NQS species.

*Injection volume*

The effect of the injection volume on both peak area and resolution was investigated. The samples were injected at a constant pressure at 0.5 psi; thus, a variation in the injection time led to a variation in the injection volume. In this study, injection times between 1 and 25 s were used, which corresponded to injection volumes between 4.5 and 112.5 nL. Results show that the resolution was dramatically lowered with increasing injection time. In this way, for injection times longer than 10 s, the peaks of most amino acid derivatives overlapped. An injection time of 5 s was finally

chosen as a suitable compromise between sensitivity and resolution.

*Temperature*

The effect of the temperature of the capillary on the separation of amino acid derivatives was studied in the range of 15 to 35°C. The resolution was slightly improved by decreasing temperature, but at the same time, a greater increase in the migration times was observed. Taking into account both resolution and analysis time, a temperature of 25°C was chosen for further experiments.

*Potential*

The working potential was optimized for the 40mM sodium tetraborate–isopropanol (3:1, v/v) hydroorganic solution. This study showed a continuous increase in the migration velocity with potential, and at the same time, a slight improvement in the resolution was attained. The final potential chosen was 30 kV, which generated current intensities of approximately 55 µA.

For a quicker analysis of the acidic amino acids (Glu, Asp, and Cys), a 20mM sodium tetraborate aqueous solution was used. In this case, the recommended potential was 15 kV in order to avoid overheating and forming bubbles in the capillary. Figure 3 shows the electropherograms of the separation of amino acid derivatives using these aqueous and hydroorganic background electrolyte solutions.

The separation of partially resolved peaks can be improved at the expense of increasing the analysis time. Experimentally, this could be achieved by slightly increasing the percentage of organic solvent and the concentration of buffer in the support solution. Conversely, faster separations also led to poorer resolutions. As a result, the selected experimental conditions aim to achieve a suitable compromise between resolution and analysis time.

**Figures of merit**

Table I shows the characteristics of the method for various amino acids. Although both peak area and peak height can be used for quantitative purposes, preliminary studies showed that the best precision was provided using the peak area. Hence, this parameter was chosen to establish the figures of merit of the present method. Detection at 480 nm was preferred in terms of robustness and precision, even though the sensitivity at 305 nm was approximately 3 times higher than that reached at 480 nm.

The repeatability of peak area and retention time on the same day (i.e., run-to-run precision) was calculated as the relative standard deviation (RSD) of 6 consecutive injections of a standard solution with a concentration of $6.6 \times 10^{-4}$ M of every amino acid. The limit of detection (LOD) was calculated for a signal-to-noise ratio of 3. In summary, repeatabilities of peak areas were approximately 5%, and repeatabilities of migration times were generally approximately 3%. The linear range for the amino acids was checked up to $3.2 \times 10^{-3}$ M, and detection limits ranged between 70 and 850 amol when injecting for 5 s.

Typical ranges of parameters for other UV–visible CE methods described in the literature (3,25,26) in terms of LOD, analysis time, and resolution are given for the sake of comparison with the present method: LODs ranged between 150 amol and 6 fmol, and analysis times ranged between 30 and 60 min. Additionally, a complete resolution of the peaks of common amino acids (e.g., 20 proteic amino acids) was difficult, and poorly resolved peaks for some amino acid derivatives often arose in the electropherograms.

**Determination of lysine in pharmaceutical samples**

The proposed method was applied to the determination of amino acids contained as dietary supplements in several pharmaceutical samples. Because the matrix composition of these samples modified the sensitivity of the calibration, a standard addition method was required to quantitate the lysine content. This matrix effect was mainly attributed to the presence of acidic compounds that lowered the pH in the reaction medium and, consequently, may have affected the yield of the derivatization procedure.

**Table I. Figures of Merit of the Proposed CE Method at 480 nm Stabilized Under Optimal Working Conditions**

| Amino acid | Migration time (min) | repeatability (%RSD) | Retention time repeatability (%RSD) | Peak area Straight line equation* | Correlation coefficient | Detection limit (pmol) |
|---|---|---|---|---|---|---|
| Lys† | 30.4 | 0.54 | 3.0 | $A = 7 \times 10^3 c + 5.7$ | 0.983 | 0.67 |
| Trp† | 34.8 | 1.60 | 4.9 | $A = 2 \times 10^4 c + 12.3$ | 0.979 | 0.22 |
| Leu† | 39.1 | 1.77 | 4.5 | $A = 4 \times 10^4 c + 28.2$ | 0.996 | 0.11 |
| Ilea† | 39.5 | 1.79 | 4.6 | $A = 3 \times 10^4 c + 9.4$ | 0.995 | 0.16 |
| Tyr† | 40.6 | 1.22 | 3.2 | $A = 7 \times 10^3 c + 2.8$ | 0.974 | 0.67 |
| Val† | 42.3 | 1.86 | 4.2 | $A = 10^4 c + 9.8$ | 0.967 | 0.45 |
| Hyp† | 43.8 | 2.04 | 6.5 | $A = 4 \times 10^4 c + 0.49$ | 0.902 | 0.11 |
| Ala† | 46.1 | 2.06 | 8.5 | $A = 6 \times 10^4 c - 5.6$ | 0.979 | 0.07 |
| Thr† | 61.8 | 3.22 | 6.9 | $A = 5 \times 10^4 c + 2.7$ | 0.992 | 0.10 |
| Gly‡ | 15.6 | 7.22 | 7.2 | $A = 10^5 c + 8.4$ | 0.991 | 0.58 |
| Glu‡ | 25.5 | 12.1 | 5.3 | $A = 1 \times 10^5 c + 71$ | 0.995 | 0.36 |
| Asp‡ | 29.0 | 13.1 | 6.1 | $A = 4 \times 10^5 c + 14$ | 0.998 | 0.85 |
| Cys‡ | 30.2 | 14.1 | 5.8 | $A = 4 \times 10^3 c + 0.3$ | 0.996 | 0.55 |

* $c$ is concentration (M), $A$ is absorbance (mAU).
† 40mM sodium tetraborate–isopropanol (3:1, v/v) background electrolyte solution at 30 kV.
‡ 20mM sodium tetraborate background electrolyte solution at 15 kV.

Figure 4 shows (as an example) the electropherogram obtained for a sample solution of Tebetane. In order to test the accuracy of the proposed method, the results were compared with those from the standard method (23) for amino acid analysis (Table II). All samples were analyzed in triplicate; good concordance between both methods was observed, with an overall quantitation error of 12.3% for the amino acids present in the samples.

## Conclusion

The present paper describes a feasible CE method based on precapillary derivatization with NQS for the determination of amino acids. The separation conditions were optimized to achieve the resolution of a general amino acid mixture. The method can be adapted for a faster determination of a specific amino acid of interest. When a higher resolution is needed for a particular sample, an increase in the percentage of isopropanol and a decrease in the capillary temperature can be employed. The accuracy in the determination of amino acids in the pharmaceutical samples shows the validity of the method for the analysis of real samples. This method can be easily extended to other kinds of application in the field of amino acids. In addition, this reaction can be applied to other types of compound, because NQS is a general reagent of primary and secondary amino groups.

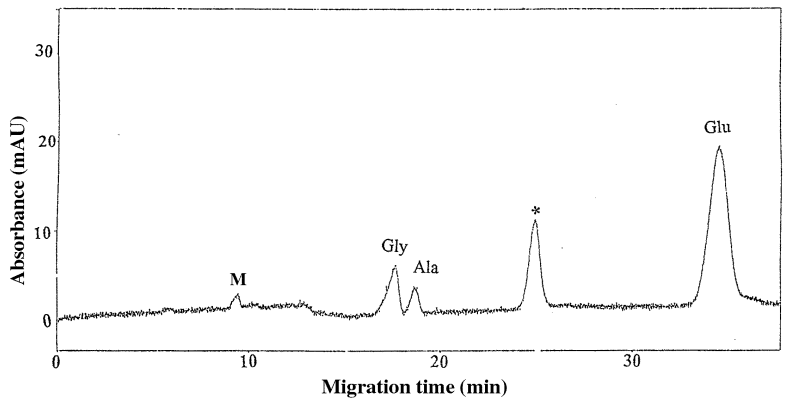


**Figure 4.** Electropherogram obtained for the separation of amino acid derivatives of a sample solution of Tebetane. Electrophoretic conditions: background electrolyte solution, 20mM sodium tetraborate (pH 9.2); potential, 15 kV; injection time, 5 s; temperature, 25°C. Reaction conditions: time, 5 min; temperature, 65°C; reagent solution, $3 \times 10^{-2}$ M NQS–0.1M HCl; buffer solution pH, 10; volume of reagent, buffer, and sample solution, 250 µL each. M indicates the marker. The asterisk (*) indicates an NQS species.

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**Table II. Determination of Amino Acids in Pharmaceutical Samples by Using the Proposed Method (Aqueous CE Conditions) and the Standard Method**

| | Pranzo* | | Troforex pépsico* | | Tebetane | | Trimetabol | | Salvacolon* | | Policolinosil* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid | CE method | Standard method | CE method | Standard method | CE method | Standard method | CE method | Standard method | CE method | Standard method | CE method | Standard method |
| Lys | 0.90 | 0.99 | 2.47 | 2.40 | — | — | 4.75 | 4.43 | 6.48 | 5.41 | 0.13 | 0.18 |
| Glu | — | — | — | — | 2.53 | 2.49 | — | — | — | — | — | — |
| Gly | — | — | — | — | 0.59 | 0.54 | — | — | — | — | — | — |
| Ala | — | — | — | — | 0.88 | 0.72 | — | — | — | — | — | — |

* Expressed as grams amino acid per 100 mL sample solution.
† Expressed as grams amino acid per capsule.
‡ Expressed as grams amino acid per tablet.

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