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Continuous flow derivatization system coupled to capillary electrophoresis for the determination of amino acids

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Abstract

A derivatization system coupled to capillary electrophoresis for the determination of amino acids using 1,2-naphthoquinone-4-sulfonate as a labeling agent is described. In this system, amino acids are derivatized on-line in a three-channel flow manifold for sample, reagent and buffer solutions. The reaction takes place in a PTFE coil heated at 80 °C. The resulting solution, which contains the amino acid derivatives, is introduced into the electrophoretic system by means of an appropriate interface. Subsequently, amino acid derivatives are separated at 25 kV using a 40 m*M* sodium tetraborate aqueous solution with 30% (v/v) isopropanol solution as a running buffer. The electropherograms are monitored spectrophotometrically at 230 nm. The method has been applied to the determination of amino acids in feed samples and pharmaceutical preparations. A good concordance of the predicted values with those given by a standard amino acid analyzer is shown.

Keywords: Continuous flow derivatization; Derivatization, electrophoresis; Amino acids; Naphthoquinonesulfonate

1. Introduction

Derivatization procedures are commonly applied in combination with separation techniques to enhance the detection sensitivity. This is particularly relevant in capillary electrophoresis (CE) as the narrow optical path and internal diameter of the capillary lead to poor sensitivities. Other important goals of derivatization in CE are the improvement of detection selectivity, the incorporation of a charge to neutral analytes for facilitating the separation, the formation of diastereomeric derivatives for resolving chiral compounds, etc. [1].

Focusing the description on the possible derivatization modes for CE, the following approaches can be considered.

(i) Pre-capillary derivatization, in which the reaction is developed prior to the sample injection into the capillary so that the corresponding analyte derivatives are actually separated. In this case, the derivatization can be carried out following a conventional batch procedure or by coupling a flow system to the CE. To date, pre-capillary derivatization is the most popular mode for development of the reaction. Preliminary sample manipulations, including dialysis, gas diffusion, liquid–liquid extraction, solid-phase extraction, etc., can be combined with

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the derivatization procedure for sample preconcentration and clean-up and removal of the reagent excess [2,3]. Such analytical manipulations are hardly implemented in the other derivatization modes (see below, points ii and iii).

(ii) In-capillary derivatization consists of developing the reaction inside the capillary by promoting the mixing of analytes with the reagent. A remarkable feature of this technique is the small reaction chamber volume, which means that the sample consumption and dilution are minimal. In-capillary derivatization can be accomplished by different strategies such as zone-passing [4–6], at-inlet [7] and throughout capillary derivatization [8,9].

(iii) Post-capillary derivatization is based on sample injection directly into the capillary so that the underivatized analytes are separated. Subsequently, the on-line derivatization reaction is developed prior to the detection of the corresponding derivatives. Post-capillary approaches can be based on gap designs [10], permeable membranes [11] and new chip technologies [12].

As indicated above, off-line sample pretreatments are commonly used before the sample injection into the CE system. However, off-line manipulations may involve a lack of precision, time consumption and manual handling of toxic reagents and organic solvents. Continuous flow systems are powerful tools for the implementation of the preliminary operations of the analytical process. The principal advantage of flow systems is the possibility of designing a specific set-up for each particular application. Flow procedures open up the possibility of automation simplification and miniaturization in a wide variety of sample pretreatments of routine application. Automated procedures lead to better precision and higher sample throughput, and decrease the consumption of sample and reagents. The combination or hyphenation of flow methodologies with separation techniques seems to be an attractive way to enhance the analytical potential providing more robust and reliable methods [2,3].

The main problem in this coupling arises in the design of a suitable interface to make compatible the typical flow-rates of flow systems with the low injection volumes required in CE. An exhaustive revision of unconventional samplers and interfaces for hyphenation of flow systems such as flow injection analysis (FIA) and HPLC with CE is presented in Ref. [13]. The pioneering systems using gaps and special injection devices were proposed at the beginning of the 1990s [14,15]. More recently, FIA–CE systems developed by Kuban et al. allow consecutive runs to be injected [16,17]. The analytical possibilities of this coupling have been proven in examples such as the separation of small ions in complex food matrices [18,19]. In some cases, the sample treatment involved on-line gas diffusion for removal of the sample matrix [20]. FIA–CE systems can be combined with a stacking process for an additional enhancement of the sensitivity [21]. Fang et al. have proposed a combined FIA–CE system using conventional equipment. The interface is based on a conical inlet which connects the FIA manifold with the CE capillary [22]. Valcárcel's group has developed an interface consisting of a robotic arm which introduces a discrete volume of pretreated sample into the CE instrument. For instance, such a device has been applied to the determination of anti-inflammatory drugs in biological fluids [23], polyphenolic species [24] and inorganic ions in waters [25]. Other authors have reported a gradient elution system for electrochromatography based on an FIA interface [26].

In a previous paper, a CE method for the determination of amino acids based on a pre-capillary derivatization with 1,2-naphthoquinone-4-sulfonate (NQS) was proposed [27]. Here, a new hyphenated procedure to carry out this derivatization using a continuous flow system (CFA) is developed. The derivatization process occurred in the flow manifold by mixing the analytes with NQS. Subsequently, amino acid derivatives were introduced in the sample vial which was designed for operating as a continuous flow interface. The sample was injected electrokinetically by applying 10 kV. Amino acid derivatives were separated in a fused-silica capillary with a sodium tetraborate electrolyte buffer using 2-propanol as an organic modifier. This technique results in a straightforward and automated way of carrying out the derivatization. Additionally, the approach is more attractive in comparison with conventional batch procedures in terms of reproducibility and minimization of side reactions. The method was applied to the determination of amino acids in feed and pharmaceutical samples. Results show a good

concordance with those given by a standard amino acid analyzer.

2. Experimental

2.1. Reagents and solutions

Unless specified, all reagents were of analytical grade. Solutions were prepared with Milli-Q (Millipore, Milford, MA) water. The running buffer consisted of 40 m*M* sodium tetraborate aqueous solution (Carlo Erba, Milan, Italy)–isopropanol (HPLC grade, Panreac, Barcelona, Spain) (70:30, v/v). The reagent solution was composed of 0.06 *M* NQS (Carlo Erba)–0.1 *M* HCl (Merck, Darmstadt, Germany). The buffer solution for the development of the reaction was 0.05 *M* sodium tetraborate–0.1 *M* sodium hydroxide (both Merck). Amino acids were purchased from Merck.

2.2. Samples

Feed samples and pharmaceutical products were analyzed in order to check the applicability of the present CFA–CE method. More information regarding these samples and the sample treatments are given below.

Commercial feed samples for animal nutrition were kindly provided by Cooperativa Agropecuaria de Guissona, Lleida, Spain. The analytical interest was focused on the quantification of amino acids contained in protein hydrolysates. The hydrolysis consisted of the treatment of 1 g of feed product with 4 ml of 6 *M* HCl for 24 h at 105 °C. This procedure was carried out in a 10-ml glass-stoppered vial using a heating aluminum block. The content of the vial was neutralized with sodium hydroxide, diluted with water to 10 ml and filtered through a 45-μm pore size cellulose acetate membrane (Schleicher and Schuell, Dassel, Germany). A further 50-fold dilution with water was performed before the amino acid derivatization in the CFA system.

The pharmaceutical samples consisted of preparations containing several amino acids which were used as a dietary supplement for stress and malnutrition. Aminoveinte (suspension, Laboratorios Madariaga, Alcorcón, Madrid, Spain) was diluted 5-fold with water and filtered through a 45-μm pore size cellulose acetate membrane. Marlidan sample (solution, Laboratorios Viñas, Barcelona, Spain) was diluted 50-fold with water and filtered through a 45-μm pore size cellulose acetate membrane. In the case of Neocate (powdered milk, SHS International, Liverpool, UK), 0.1 g of powdered product was treated with 25 ml of water. The suspension obtained was filtered through a nylon membrane of 0.45-μm pore size. In all cases, the resulting sample solutions were used directly for the CFA–CE analysis.

2.3. Apparatus

A P/ACE Beckman capillary electropherograph with a diode array spectrophotometric detector was used. Electropherograms were monitored at 230 nm and processed with a compatible computer using the Beckman P/ACE station (version 1.0) software. Fused-silica capillaries (supplied by Tecknokroma) of 75 μm I.D.×375 μm O.D. with an effective length of 58.7 cm and total length of 67 cm were used. A Pharmacia LKB autoanalyzer (model Alpha Plus, series 2) was used for the standard determination of amino acids based on a cation-exchange separation and post-column derivatization with ninhydrin.

2.4. Derivatization system

The continuous flow derivatization system consisted of a three-channel manifold as shown in Fig. 1. Solutions were pumped with a peristaltic pump (Watson-Marlow 505 DU) using standard Tygon tubing (Cole-Palmer). T-Pieces and connectors were made of PTFE. Reagent and buffer solutions were joined and mixed in a 2 m×0.5 mm I.D. PTFE coil. The derivatization process occurred when mixing the resulting reagent solution with the sample stream in a PTFE reaction coil (3 m×1.1 mm I.D.) heated at 80 °C using a thermostatic bath. The resulting derivatized sample solution was continuously introduced into the injection vial which was set in the autosampler carousel of the CE instrument. The level of liquid in this vial was controlled by means of a waste channel which aspirated the spare solution. Flow rates were 0.25 ml min^{-1} for each sample, reagent and buffer channel and 1 ml min^{-1} for the waste

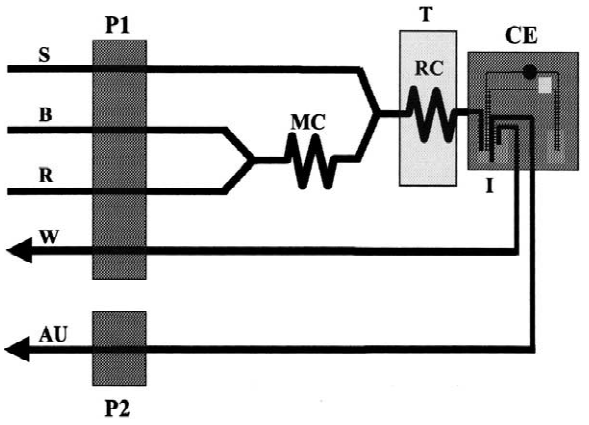

Fig. 1. Scheme of the continuous flow derivatization–capillary electrophoresis system (CFA–CE). AU, auxiliary channel; B, buffer channel (0.05 *M* sodium tetraborate–0.1 *M* sodium hydroxide); CE, capillary electrophoresis; I, continuous flow interface; MC, mixing coil (2 m×0.5 mm I.D.); P1, peristaltic pump; P2, auxiliary peristaltic pump (for emptying the system); T, thermostatic bath (80 °C); R, reagent channel (0.06 *M* NQS–0.1 *M* HCl); RC, reaction coil (3 m×1.1 mm I.D.); S, sample channel; W, waste channel. Flow rate, 0.25 ml min^{-1} for channels S, B and R; 1 ml min^{-1} for channel W; 2 ml min^{-1} for channel AU.

channel. An additional channel for emptying out the interface vial quickly was used to totally empty the content of this vial. This channel operating at 2 ml min^{-1} was activated after the sample injection into the CE system. Conversely, the channel was stopped while derivatization was developed so that the interface was filled again with the solution containing the derivatives. In this way, the CE system was ready to analyze a new sample solution.

2.5. Capillary electrophoresis conditions

Amino acid derivatives were injected into the capillary electrokinetically by applying 10 kV for 25 s. The separation was carried out at 25 kV and 25 °C using a running buffer consisting of 40 m*M* sodium tetraborate aqueous solution (pH 9.2)–isopropanol (30:70, v/v). A forward pressure of 0.5 p.s.i. (1 p.s.i.=6894.76 Pa) was applied 45 min after injection in order to accelerate the migration of the acidic amino acids (glutamic acid, aspartic acid and cysteine).

3. Results and discussion

3.1. Optimization of continuous-flow derivatization conditions

In the optimization studies described below various amino acids were chosen as model cases as follows. Glycine and phenylalanine were examples of polar and non-polar amino acids; glutamic acid was selected as an acidic amino acid; lysine was representative of the basic amino acid group; and proline was a secondary amino acid.

3.1.1. Temperature

The effect of temperature on the development of the reaction was studied in the range 40–90 °C by varying the temperature of the thermostatic bath in which the reaction coil was immersed. Results obtained are shown in Fig. 2 for representative amino acids selected. The temperature influences in opposite ways: (i) the formation of derivatives is accelerated as the reaction is a kinetic (temperature dependent) process; (ii) reagent and derivatives may undergo progressive degradation, especially at high temperature. Most of the amino acids showed an increase in the peak areas of the corresponding derivatives that was directly proportional to the temperature. In particular, the low yield of the

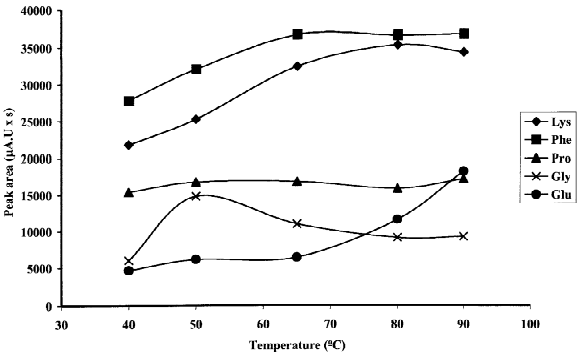

Fig. 2. Effect of temperature on the development of the reaction. Derivatization conditions: reagent solution, 0.06 *M* NQS–0.1 *M* HCl; buffer solution, 0.05 *M* sodium tetraborate–0.1 *M* sodium hydroxide; reaction coil, 2 m×1.1 mm I.D.; total flow-rate, 1.5 ml min^{-1}. Separation conditions: running buffer, 40 m*M* sodium tetraborate–isopropanol (20% v/v); running voltage, 20 kV; sample injection, 7 kV for 10 s.

reaction for acidic amino acids (below 70 °C) was increased significantly at higher temperatures. The reaction of NQS with proline was faster so that this compound can be derivatized even at low temperature. For glycine, the sensitivity increased with temperature in the range 40–50 °C but decreased at higher values. Finally, the temperature selected was 80 °C as a reasonable compromise for all amino acids.

3.1.2. NQS concentration

The influence of NQS concentration on the development of the reaction was studied in the range 0.01–0.1 *M*. All NQS solutions were prepared in 0.1 *M* HCl. For most of the amino acids, a plateau zone was achieved for NQS concentrations in the range 0.03–0.1 *M*. Conversely, acidic amino acids (e.g. Glu) needed high NQS concentrations to increase the yield of the derivatization although a high excess of free reagent led to broad NQS peaks which may overlap and interfere with derivatives, and additional peaks related to side products and decomposition species may appear. In consequence, the NQS concentration selected as optimal was 0.06 *M*. This provided a suitable compromise between sensitivity and interference from NQS excess and side products.

3.1.3. Residence time

The reaction time between amino acids and reagent in the continuous flow manifold can be controlled as a function of the flow-rate and the reactor dimensions (length and inner diameter). Hence, these parameters were optimized in order to achieve a suitable derivatization as well as minimizing side reactions. The influence of total flow-rate was evaluated in the range 0.75–2.25 ml min^{-1} for a reaction coil of 4.5 m×0.5 mm I.D., which corresponded to residence times between 1.2 and 0.4 min. For each case, the flow-rate of each individual channel was one-third of the total flow-rate. As shown in Fig. 3, the development of the reaction increased inversely with the total flow-rate (i.e. increasing the residence time), and a value of 0.75 ml min^{-1} was chosen as optimal for this variable.

The length of the reaction coil for the development of the derivatization process was varied from 1 to 9 m. In all cases the inner diameter was 1.1 mm and

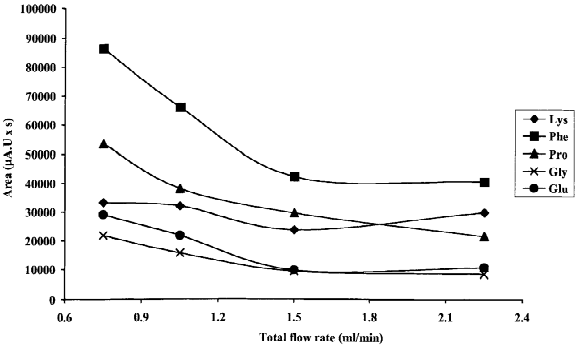

Fig. 3. Effect of the total flow-rate on the development of the reaction. Derivatization conditions: reagent solution, 0.06 *M* NQS–0.1 *M* HCl; buffer solution, 0.05 *M* sodium tetraborate–0.1 *M* sodium hydroxide; reaction coil, 2 m×1.1 mm I.D.; temperature, 80 °C. Separation conditions: running buffer, 40 m*M* sodium tetraborate–isopropanol (80:20, v/v); running voltage, 20 kV; sample injection, 7 kV for 10 s.

the total flow-rate 0.75 ml min^{-1}. In the range under study, responses were approximately constant for several amino acids (e.g. Gly, Pro, Lys) which indicated that their derivatization reactions were completed even at low residence time (this corresponded to the faster kinetic processes). Conversely, other amino acids such as Glu and Phe showed an initial increase in their responses when the reactor length was increased and reached a plateau zone in the range 3–9 m. These last results show the quantitative derivatization of all analytes which is essential in pre-capillary derivatization. In conclusion, a reaction coil of 3 m×1.1 mm I.D. was sufficient to form all amino acid derivatives when using a total flow-rate of 0.75 ml min^{-1} (i.e. residence time, 1.4 min) and a 0.06 *M* NQS solution.

3.1.4. Sample injection

The selection of the injection mode was conditioned for the particular design of the continuous flow interface between the derivatization and CE systems and, in this case, an electrokinetic injection mode was chosen. The experimental parameters to be optimized in this study were the voltage and injection time. The injection voltage was varied from 3 to 10 kV (10 kV was the maximum limit available for electrokinetic injection for this Beckman instrument). The experimental results showed that corresponding peak areas for all derivatives continuously

increased when higher voltages were applied so that 10 kV was finally chosen as optimum. Subsequently, the influence of the introduction time on the sensitivity and the shapes of peaks was evaluated. The range for the study was from 10 to 40 s and the potential applied in all cases was 10 kV. An increase in the peak height in the range 10–25 s was observed for all amino acids while from 25 s the peak height remained practically constant. At the same time, peaks became dramatically wider for injection times higher than 25 s so that the peak resolution was seriously affected. Hence, the introduction time finally selected was 25 s as a suitable compromise between sensitivity and resolution.

As a summary of these continuous flow derivatization studies, all amino acids could be derivatized, some of them under mild conditions while others required more drastic conditions. Although temperature was one of the most significant parameters affecting the reaction, a quantitative derivatization of amino acids cannot be accomplished exclusively with increasing this variable as thermal degradation may occur. In consequence, moderate temperatures combined with suitable residence time were chosen to enhance the reaction yield while minimising side processes. As a result, the final conditions selected were those that provided a complete derivatization of all amino acids with a negligible degradation of derivatives.

3.2. Study of the separation conditions

The separation of NQS amino acid derivatives was optimized elsewhere [27]. In principle, the separation should be independent of the way of obtaining the derivatives (i.e. either batch or flow derivatization). Thus, the separation conditions described in Ref. [27] were the basis of the present study. Here, the most relevant modification in the electrophoretic conditions was the application of a positive pressure of 0.5 p.s.i. 45 min after injection. This step accelerated the migration of acidic amino acids, speeding up the analysis. In this way, basic, neutral and acidic amino acids were separated and analyzed in the same electrophoretic run. Another advantage of the CFA–CE system proposed here with respect to electropherograms obtained in the batch derivatization, was the minimization of the side and decomposition reactions. As a result, electropherograms from the on-line continuous flow derivatization displayed simpler and clearer profiles.

3.3. Figures of merit

Figures of merit of the CFA–CE method were established under the selected experimental conditions described above. The peak area was selected as a quantitative descriptor to be correlated with the analyte concentration. The dynamic range was studied from $1.6 \cdot 10^{-5}$ to $4 \cdot 10^{-4}$ M of each amino acid. In all cases, good linearity was obtained with correlation coefficients higher than 0.99. The repeatability in the migration time and peak area was evaluated at a concentration level of $1.28 \cdot 10^{-4}$ M for each amino acid from a series of six successive injections of an amino acid standard solution. As shown in Table 1, the repeatability in the migration time was better than 1.5% and it was ~3–10% for peak areas. The detection limit was in the range from 0.39 to 8.48 mg l^{-1}.

In comparison with the batch derivatization procedure, the precision for the CFA–CE method for some of the amino acids was better as well as the linearity. Moreover, the detection limit of some of the amino acids limit was enhanced 10-fold. Improvements in the analytical characteristics of this new method were attributed to the feasibility and robustness of the on-line derivatization with respect to the manual procedure.

3.4. Determination of amino acids

The CFA–CE method proposed was applied to the determination of amino acids in two types of samples, namely: (i) protein hydrolysates of feed samples for feeding animals in which the content of essential amino acids is indicative of the nutritional value; (ii) pharmaceutical preparations containing several amino acids which are mainly used as dietary supplement for humans when their amino acid needs are greatly enhanced (e.g. in severe physical or pathological proteic stress). The sample volume required for the analysis using the continuous flow derivatization system is ~5 ml. However, this is not a

Table 1
Figures of merit of the proposed method

Amino acid	Migration time (min)	Migration time repeatability (RSD, %)	Peak area repeatability (RSD, %)	Straight line equation[a]	Correlation coefficient	Detection limit (mg l^{-1})
Lys	32.8	1.0	4.9	$1.78 \times 10^5 c + 0.26$	0.998	5.48
Orn	35.2	0.9	4.2	$1.26 \times 10^5\ c + 0.82$	0.999	5.06
Trp	36.2	1.0	6.8	$8.45 \times 10^5 c + 9.98$	0.998	0.61
Phe	39.0	1.0	3.3	$1.53 \times 10^5 c + 15.3$	0.999	0.50
Leu	39.5	1.0	5.3	$1.90 \times 10^5 c + 19.5$	0.997	0.39
Ile	40.0	1.1	8.2	$4.45 \times 10^5 c + 6.38$	0.999	2.10
Met	41.7	1.1	5.2	$4.34 \times 10^5 c + 9.53$	0.995	4.48
Val	42.6	1.1	5.8	$2.58 \times 10^5 c + 0.22$	0.999	1.87
Hyp	42.7	1.1	2.6	$3.73 \times 10^5 c - 9.32$	0.994	2.63
Gln	43.1	1.1	2.7	$7.19 \times 10^4 c + 1.50$	0.995	5.85
Asn	44.3	1.1	3.5	$1.62 \times 10^5 c + 2.21$	0.998	4.76
Thr	44.8	1.2	5.8	$2.46 \times 10^5 c + 1.62$	0.998	2.98
Pro	46.1	1.1	2.9	$3.12 \times 10^5 c + 1.77$	0.999	2.30
Ser	48.2	0.9	4.7	$9.64 \times 10^4 c + 2.94$	0.998	2.10
Gly	49.7	0.3	7.0	$4.68 \times 10^4 c + 1.54$	0.994	3.00
Glu	53.3	0.4	9.6	$3.20 \times 10^5 c + 1.12$	0.993	5.89
Asp	59.5	0.4	9.7	$3.12 \times 10^5 c + 9.60$	0.994	7.99
Cys	60.2	0.5	9.9	$6.04 \times 10^4 c + 35.1$	0.999	8.48

[a] c, concentration in mol l^{-1}; peak area expressed in mAU s^{-1}.

critical parameter as large sample volumes were available for the pharmaceutical and feed samples analyzed.

Results obtained are given in Tables 2 and 3 for feed samples and pharmaceutical products, respectively. Examples of electropherograms of a standard solution and a pharmaceutical and a hydrolysate samples are shown in Figs. 4 and 5. In order to evaluate the accuracy of the proposed method, these results were compared with those obtained with the standard method for amino acid analysis. The standard procedure was based on ionic chromatographic separation of amino acids in a sulfonic analytical column and further post-column derivatization with ninhydrin. Overall prediction errors were ~5%. Therefore, a reasonable concordance between the amino acid amounts given by the CFA–CE and the standard method was found in all cases. This suggested that the proposed method may be an attractive alternative to the standard analysis. The main advan-

Table 2
Determination of amino acids in feed samples using the proposed CFA–CE method

Amino acids	Feed sample A		Feed sample B		Feed sample C	
	CFA–CE method (%)	Standard method (%)	CFA–CE method (%)	Standard method (%)	CFA–CE method (%)	Standard method (%)
Lys	0.47	0.53	0.55	0.48	0.15	0.13
Phe	0.46	0.41	0.49	0.31	0.31	0.16
Ile	0.29	0.21	0.35	0.27	0.11	0.11
Val	0.38	0.37	0.49	0.37	0.18	0.16
Thr	0.31	0.31	0.42	0.37	0.17	0.17
Pro	0.41	0.38	0.50	0.46	0.33	0.33
Gly	0.17	0.13	0.32	0.30	0.19	0.19
Glu	1.26	1.14	1.34	1.41	0.79	0.78
Asp	0.64	0.62	1.33	0.64	0.57	0.54

Table 3
Determination of amino acids in pharmaceutical preparations

Amino acid	Amino20[a]		Marlidan[a]		Neocate[b]	
	CFA–CE method	Standard method	CFA–CE method	Standard method	CFA–CE method	Standard method
Thr	–	–	51.22	44.31	0.86	0.72
Gly	4.48	4.09	313.79	275.5	1.04	0.98
Val	–	–	38.42	35.44	1.21	1.30
Met	51.03	52.67	124.88	126.67	0.33	0.31
Ile	–	–	22.69	19.94	1.11	1.15
Phe	–	–	82.10	79.71	0.86	0.76
Lys	17.83	22.65	273.98	327.40	1.75	1.50
Trp	–	–	–	–	0.35	0.34
His	6.78	6.69	–	–	–	–

[a] Expressed as mg per 100 ml of sample emulsion.
[b] Expressed as g per 100 g of sample.

tages of this CE method with respect to the standard counterpart lie in the significant (3-fold) decrease in the analysis time and the significant reduction in the consumption of reagents.

4. Conclusions

The coupling of techniques is one of the fields of analytical chemistry currently receiving a great deal

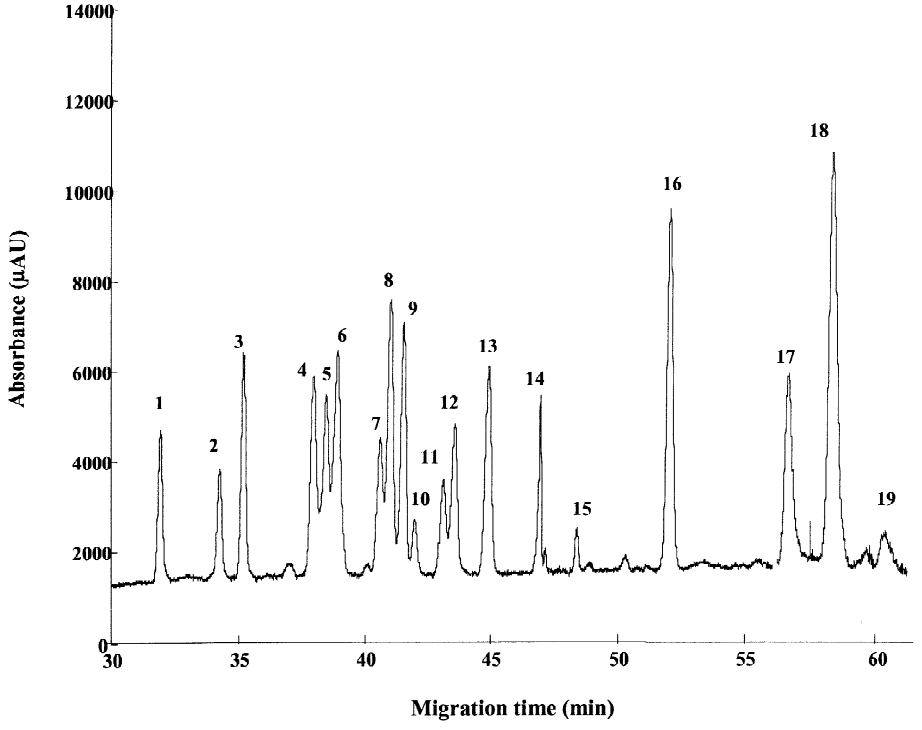

Fig. 4. Electropherogram of a standard amino acid solution under the experimental conditions selected. Peak assignment: 1=Lys, 2=Orn, 3=Trp, 4=Phe, 5=Leu, 6=Ile, 7=Met, 8=Val, 9=Hyp, 10=Gln, 11=Asn, 12=Thr, 13=Pro, 14=Ser, 15=Gly, 16=NQS, 17=Glu, 18=Asp, 19=Cys.

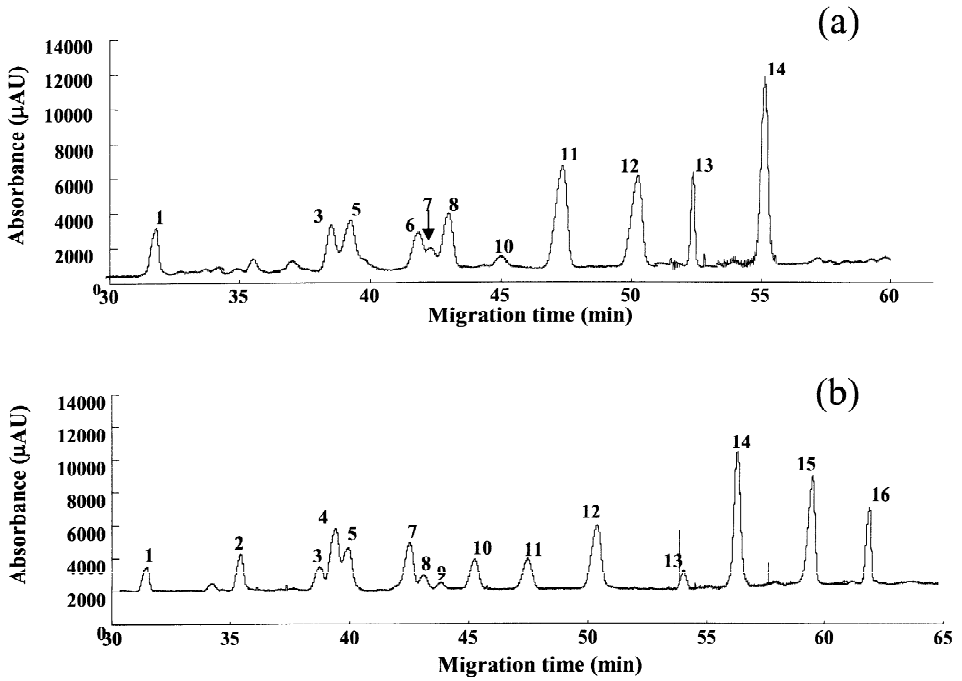

Fig. 5. Electropherogram of a pharmaceutical sample (a) and feed sample hydrolysate (b) obtained under the experimental conditions selected. Peak assignment: 1 = Lys, 2 = Orn, 3 = Phe, 4 = Leu + His, 5 = Ile, 6 = Met, 7 = Val, 8 = Hyp, 9 = Gln, 10 = Thr, 11 = Pro, 12 = Ser + Ala, 13 = Gly, 14 = NQS, 15 = Glu, 16 = Asp.

of attention. Beyond the most classical combinations, which involve a separation technique coupled to powerful spectrometric techniques, other possibilities are being investigated to increase the analytical potential of hyphenated systems. Here, the CFA–CE method proposed has proven its feasibility for the analysis of amino acids with an on-line derivatization procedure. The resulting hyphenation between derivatization and separation systems takes advantage of synergistic effects which are not reached when the two systems operate separately. Hence, this method is focused on solving some general drawbacks of off-line sample treatment and derivatization which are tedious, time-consuming and may be the main source of random and systematic errors. Another specific goal concerning the determination of amino acids with NQS is the minimization of the degradation of derivatives and reagent. Furthermore, the sensitivity can be improved and the resulting electropherograms are freer of ghost peaks associated with side products. Further studies are in progress to develop a new interface system for implementing this derivatization with a flow injection manifold to decrease the sample volume.

Acknowledgements

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