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Multiple injection technique for the determination and quantitation of insulin formulations by capillary electrophoresis and time-of-flight mass spectrometry

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ABSTRACT

This paper describes an efficient CE-UV-ESI-TOF/MS method for the determination and quantitation of intact insulin (INS) in a pharmaceutical formulation. The CE conditions were optimized to avoid the adsorption of proteins onto the capillary wall. Particular attention was paid regarding the choice of the internal standard (IS). A strategy based on multiple injections was selected and the methodology was validated according to international guidelines. The optimized method was applied with success to the analysis of INS formulations obtained from regular and parallel markets.

1. Introduction

In the pharmaceutical area, recombinant proteins produced by biotechnology have grown considerably with a market evaluated at over $70 billion *per* year by a 2010 estimate [1]. These proteins comprise antibodies, hormones, biological response modifiers to stimulate cell growth, enzymes, and vaccines [2,3]. During the biopharmaceutical development process, several parameters are needed for regulatory purposes, regarding the identity, quantity (concentration), quality, and purity of the products [4,5]. Determining the identity and concentration of the therapeutic proteins is also important after their release on the market from a quality control perspective. Since unofficial channels exist to obtain these products without prescription or without extensive evidence for quality control, therapeutic protein analysis is also relevant for the parallel market. Moreover, biopharmaceuticals available in the parallel market can be counterfeit drugs. These include products with or without the correct ingredients, without the active ingredients, with insufficient or too much active ingredients, or with fake packaging [6]. Consequently, from public health perspective, it is essential to develop analytical methods to quickly monitor the identity, quality, and quantity of these biopharmaceuticals.

For the identification and quantitation of protein formulations, the analysis of proteins in their intact form is a promising approach because no tedious sample preparation, such as a digestion step, is required. Various methods for determining the quantity of intact protein exist, and the choice of the assay mainly depends on parameters such as the quantity of protein available or the required throughput [7]. Commonly, these assays are based on UV–VIS spectroscopy (e.g., UV absorbance at 280 nm, Bradford protein assay, Lowry assay) or fluorescent detection after derivatization with a fluorescent probe (e.g., fluorescamine, 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde) [7,8]. The lack of specificity is the main bottleneck of these assays. In the contrary, mass spectrometry (MS) allows for a higher level of selectivity; often, confirmation of the product's identity is obtained through the accurate determination of its molecular mass, when high resolution mass analyzers are used [4]. To perform the simultaneous identification and quantitation of the active protein in its intact form, whether in a pharmaceutical formulation or in another matrix, it is necessary to couple a separation technique to an appropriate detector. Therefore, the hyphenation of capillary electrophoresis (CE) and MS *via* an electrospray ionization (ESI) source is an attractive option [9,10]. CE offers high speed, great efficiency, and low solvent and sample consumptions, while MS provides selectivity, sensitivity, and specificity. Due to its high mass range and mass accuracy, the time-of-flight (TOF) analyzer is particularly well suited for the detection of intact proteins that are multicharged as a result of ESI [11].

Capillary zone electrophoresis (CZE) is widely used given its versatility and compatibility with ESI-MS. However, the analysis of proteins by CZE is often impaired by the tendency of the proteins to adsorb onto the negatively charged surface of fused silica (FS) capillaries [12,13], thus degrading CE performance. The evaluation of protein adsorption and its prevention must be considered during the analytical method development, particularly when accurate

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quantitation is attempted. The choice of the internal standard (IS) is an important point to consider in quantitative analysis. Even if matrix effects seem negligible in the case of pharmaceutical formulations analysis involving good separation of active ingredient(s) and excipient(s), stable isotopically labeled (SIL) compounds and structural analogues remain the gold standards. However, for intact proteins, SIL compounds are not commonly available and/or could be very expensive [14,15]. Structural analogues differ from the intact protein by an exchange or removal/addition of amino acids, or a small modification in one or more side chains. These analogues are not easy to obtain for all proteins, can be expensive, and may present a different ionization behavior than that of the protein of interest. An alternative methodology to the IS concept was adapted from the multiple injection technique [16]. Initially developed to reduce the analysis time, this technique could be used to overcome the lack of satisfactory IS for intact proteins. In this approach, two injections are performed in the same analytical run, the first one with a standard of the protein of interest at a known concentration and the second one with the protein to be quantified. Therefore, the IS would be a standard of the protein, considered as the reference material.

In this study, a CE-UV-ESI-TOF/MS method was developed for the analysis of a recombinant human insulin (INS) as a model protein. INS was selected because of the numerous pharmaceutical formulations available on the market. Furthermore, since 1999, INS has been prohibited in sports for athletes who do not suffer from diabetes mellitus [17]. In addition, patients with this chronic disease often buy INS online without prescription because of the potentially lower cost. Due to these misuses, the risk of finding counterfeit drugs on the parallel market has increased dramatically. In the context of public health, analytical methods for quality control of these pharmaceutical formulations are needed. INS was already analyzed by CE [18–20], also coupled with MS detection [21], but never with identification and quantitation by MS. Quantitation was here attempted, using a multiple injection technique based on the successive injection of a reference standard of INS and the sample in one single run. The complete methodology was fully validated according to the guidelines of the International Conference of Harmonization (ICH) and applied to pharmaceutical formulations obtained in pharmacies and on the web without a formal prescription.

2. Material and methods

2.1. Chemicals and samples

Ammonium hydroxide solution (25%, *m*/*m*) and formic acid (98%, *m*/*m*) were of analytical grade and were purchased from Fluka (Buchs, Switzerland). Isopropanol (iprOH) and sodium hydroxide were also of analytical grade and were obtained from Acros Organics (Geel, Belgium). Acetonitrile (ACN) and methanol (MeOH) were of analytical reagent grade from Panreac (Barcelona, Spain). Ultrapure water was supplied by a Milli-Q gradient A10 purification unit from Millipore (Bedford, MA, USA). Procaïne (PROC) was obtained from Sigma–Aldrich (St. Louis, MO, USA).

The human insulin (INS) used for adsorption measurements was purchased from the National Institute for Biological Standards and Control (NIBSC, Potters Bar, United Kingdom). The protein was dissolved in a 50 mM Tris-phosphate buffer (pH 7.4) at a concentration of 0.8 mg mL^{-1} (stock solution). Standard solutions of INS of the desired concentrations were prepared daily by appropriate dilution of the stock solution with water.

Actrapid® formulations of INS (batch XS61290, Novo Nordisk A/S, Bagsvaerd, Denmark) at 100 IU mL^{-1} (stock solution), used for the validation process, were obtained from the Geneva Hospital Pharmacy (Geneva, Switzerland). Standard solutions of Actrapid®, at desired concentrations, were prepared daily by appropriate dilution of the stock solution with water. Three other batches of Actrapid® (TS62996 or batch 1, VS63735 or batch 2, and VS64228 or batch 3), used as routine samples, were obtained from the Geneva Hospital Pharmacy. One Actrapid® drug sample was purchased online (XS63170 or batch 4) without prescription.

2.1.1. Solutions used for calibration

Although it is mandatory to insure INS stability, the composition of the Actrapid® formulation (the exact nature and concentration of the excipients) was not known; for instance, the zinc and glycerine quantities were not mentioned in the manufacturer's datasheet, hindering the reconstitution of the formulation. To overcome this issue, a reference batch of Actrapid® (XS61290) was used as a stock solution and an independent sample from this batch was used on each day for the validation process (two ampoules *per* day).

The calibration function selected was the linear regression without intercept ($Y = aX$), with the target level of the calibration standard (CS) at 100%. The stock solution was diluted 20-fold to fall in the MS range and was independently prepared in three different series ($j = 3$). The CS was injected as the first injection in the multiple injections procedure (see Section 2.4). Procaine (PROC) at 50 μg mL^{-1} was added to each CS sample as an injection standard.

2.1.2. Solutions used for validation

According to the guidelines of the ICH, as well as recommendations from the "Société Française des Sciences et Techniques Pharmaceutiques" (SFSTP), three series ($j = 3$) of three independent replicates ($n = 3$) were prepared at each concentration level.

Three levels ($k = 3$) were selected for the validation standards (VS), corresponding to 75%, 100%, and 125% of the concentration. The stock solution of INS was thus diluted 25-fold, 20-fold, and 15-fold, respectively. The VS were injected as the second injection in the multiple injections procedure (see Section 2.4). Procaine (PROC) at 50 μg mL^{-1} was added to each VS sample as an injection standard.

2.1.3. Real samples

Two independent samples were prepared for each real sample by a 20-fold dilution to fall in the MS range. The CS was injected as the first injection in the multiple injections procedure (see Section 2.4). Procaine (PROC) at 50 μg mL^{-1} was added to each prepared real sample as an injection standard.

2.2. Instrumentation

2.2.1. CE system

All CE experiments were performed with an HP ^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with an on-capillary diode array detector, an autosampler, and a power supply able to deliver up to 30 kV. Separations were performed using a background electrolyte (BGE) consisting of 75 mM ammonium formate (pH 9.0) with 10% of ACN. An uncoated fused silica (FS) capillary (BGB Analytik AG, Böckten, Switzerland) was used, with an I.D. of 50 μm, a total length of 80 cm, and an effective length of 22 cm for UV detection (performed at 195 nm). The experiments were carried out in the positive polarity mode, with the anode at the inlet and the cathode at the outlet. A constant voltage of 30 kV, with an initial ramping of 5000 V s^{-1}, was applied during analysis; the capillary temperature was maintained at 25 °C. New FS capillaries were conditioned with MeOH, 1 M HCl, water, 0.1 M NaOH, water, and BGE at 1 bar for 5 min each. Prior to each sample injection, the capillary was conditioned at 2 bar for 1 min with fresh BGE. When the capillary was not in use, it was rinsed with water and dry-stored.

For area recovery experiments, an ActiPix™ D100 UV Area Imaging System (Paraytec, York, United Kingdom) was coupled with CE to perform analyses with two passes through the detector [22]. Experiments were also performed in uncoated FS capillaries with a 50 μm I.D. Capillaries with a total length of 115 cm and effective lengths of 32 and 65.5 cm were used. Samples were hydrodynamically injected at 50 mbar for 16 s (equivalent to 2% of the effective capillary length, taking into account the first window). Prior to each sample injection, the capillary was conditionned at 2 bar for 4 min with fresh BGE. UV detection was performed at 195 nm.

2.2.2. ESI-MS system

The CE instrument was coupled to a 6210 LC/MS TOF mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) *via* a coaxial sheath flow ESI interface from Agilent. The sheath liquid consisted of iprOH–water–formic acid (49.5:49.5:1, v/v/v) and was delivered at a flow rate of 4 μL min^{-1} by a syringe pump system. The ESI voltage was set at +4500 V, the nebulizing gas pressure at 4 psi, the drying gas flow rate at 4 L min^{-1}, the drying gas temperature at 150 °C, and the fragmentor voltage at 400 V. MS detection was carried out in the positive ion mode and one spectrum was acquired *per* second (9742 transients/spectrum) in the 900–2500 *m/z* range.

2.3. Adsorption measurements and calculations

Adsorption measurements were carried out according to a previous study performed in our laboratory (see Ref. [23] for experimental details).

Reversible adsorption was measured *via* the relative standard deviations of migration times (MT RSDs, $n = 5$), calculated for successive injections [24–26].

Irreversible adsorption was measured by the peak area recovery and the EOF mobility. For the area recovery, a procedure adapted from Towns and Regnier [27,28] was used. Briefly, successive injections of the protein samples were performed in the same capillary ($n = 5$) with two passes through the detector (ActiPix™ System). The decrease in area of the protein between the first and the second pass through the detector provided a measure of the irreversible adsorption on the capillary, and is given as the percentage of area recovery. Electroosmotic flow (EOF) mobilities ($n = 3$) were calculated with and without protein injection [29–31]. At a basic pH, a strong cathodic EOF occurred, and its mobility was calculated with acetone as a neutral marker. At an acidic pH, acetone migrated at around 110 min due to the weak EOF observed at this pH. Therefore, the methodology of Williams and Vigh [30] with the assistance of pressure was used.

2.4. Multiple injections procedure

The multiple injection procedure involved a series of two successive injections into the CE-UV-ESI-TOF/MS system with injection of BGE plugs in between. The CS was injected at 50 mbar for 10 s (equivalent to 0.68% of the total capillary length) as the first injection. Then, a plug of BGE was injected at 50 mbar for 130 s (8.90% of the total capillary length). The sample to be quantified was injected at 50 mbar for 10 s (0.68% of the total capillary length) as the second injection. Finally, a plug of BGE was injected at 50 mbar for 4 s (0.27% of the total capillary length).

2.5. Software

BGE solutions were prepared with the help of the PHoEBuS software (version 1.3, Analis, Namur, Belgium). CE ChemStation (version A.02.10, Agilent, Waldbronn, Germany) was used for CE instrument control. MassHunter (version B.02.00, Agilent, Waldbronn, Germany) was used for data acquisition, data handling, and spectral deconvolution.

3. Results and discussion

3.1. Method development

3.1.1. CE-ESI-MS conditions

According to previous results [23], initial experiments were carried out under acidic and basic conditions. Both acidic and basic BGE were made of 75 mM ammonium formate, at pH 2.5 and 9.0, respectively. Volatile BGEs were chosen to be directly ESI-MS compatible. Two capillaries were conditioned, and experiments were performed at each pH with and without acetonitrile (ACN) in the BGE. Different proportions of ACN were tested to improve CE performance and to reduce the adsorption of the protein onto the capillary wall. Adsorption experiments were thus performed to determine the final analytical conditions.

At an acidic pH, reversible adsorption was negligible regardless of the addition of ACN, but ACN addition was beneficial for decreasing irreversible adsorption. Globally, an acidic BGE with 10% ACN was acceptable for qualitative purposes, but in the case of quantitation, irreversible adsorption was still too important to insure acceptable repeatability. In basic conditions, there was no reversible adsorption, even in aqueous conditions; the addition of ACN eliminated irreversible adsorption. A BGE at a basic pH with 10% ACN was thus selected for the quantitative study.

ESI-MS conditions were selected according to a previous work [32], which emphasized the most important parameters for protein analysis through an experimental design. The starting conditions were finely tuned following a univariate optimization to obtain the best sensitivity for INS (estimated by the signal-to-noise ratio, S/N). To summarize, the sheath liquid made of iprOH–water–formic acid (49.5:49.5:1, v/v/v) was delivered at a flow rate of 4 μL min^{-1}, the ESI and fragmentor voltages were set at +4500 and 400 V, respectively, the nebulizing gas pressure was set at 4 psi, the drying gas flow rate at 4 L min^{-1}, and the drying gas temperature at 150 °C. Under these conditions, the limit of detection (LOD, determined for a S/N = 3) was 5 μg mL^{-1}. The response function was also evaluated over a concentration range of 5–250 μg mL^{-1}, where a linear relationship was observed.

3.1.2. Quantitative aspects

The most important issue in quantitative analysis of proteins by CE-ESI-MS is the choice of the IS, as it should feature the same ionization behavior as that of the protein to be quantified. As neither SIL nor structural analogues are easily available for all proteins and in order to obtain a methodology adaptable to several proteins, an alternative procedure was implemented. In the same run, a standard of INS at a known concentration was first injected ("IS"), followed by an injection of the sample to be quantified ("unknown"). This procedure is based on a multiple injection technique that allows the decrease of the run-to-run variability identified as a major contributor of dispersion in the results.

Initial experiments were performed with dilute Actrapid® reference solutions of 100 μg mL^{-1} for the IS and of approximately 175 μg mL^{-1} for the unknown sample. Fig. 1 shows the total ion current (TIC), the extracted mass spectrum, and the extracted ion current (EIC) for a typical CE-ESI-TOF/MS analysis obtained with this double injection approach. The $[M+3H]^{3+}$ and $[M+4H]^{4+}$ multicharged ions were detected as the major extracted ions (1937 and 1453 *m/z*, respectively). The EIC was reconstructed using both ions, and integration was achieved on the EIC. The peak area of the unknown sample was corrected by the peak area of the IS,

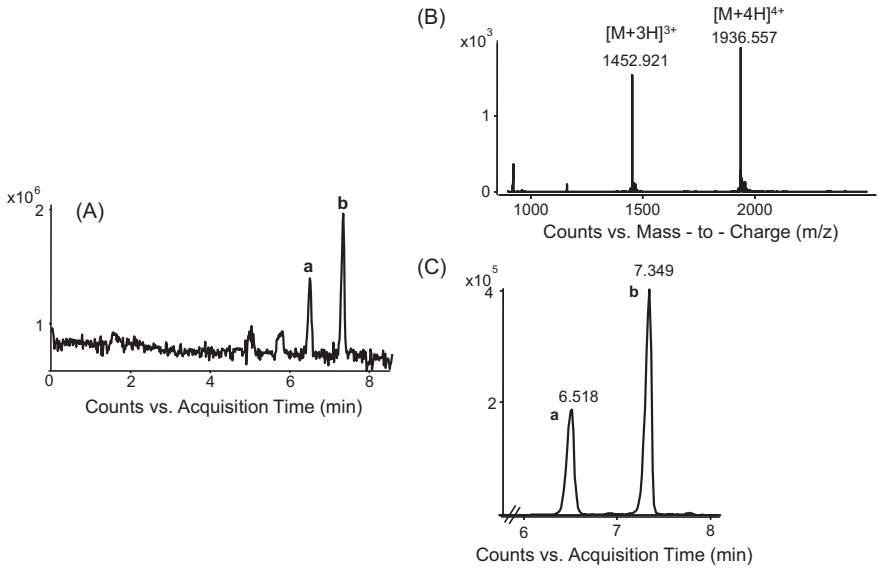

Fig. 1. CE-ESI-TOF/MS electropherograms and mass spectrum of INS obtained with the multiple injection approach: (A) total ion current (TIC), (B) extracted mass spectrum, and (C) extracted ion current (EIC). (a) IS (INS at 100 μg mL^{-1} from the first injection) and (b) unknown sample (INS at around 175 μg mL^{-1} from the second injection).

but the repeatability was not acceptable for quantitative purposes (RSD ≥ 8%, $N = 5$). Two main reasons could account for this result. Firstly, the short-term variability of ionization process could occur since both peaks did not co-migrate, as reported when structural analogues are used as IS. Secondly, the hydrodynamic injection was not repeatable enough.

Therefore, an injection standard was added to both samples (i.e., "IS" and "unknown" samples). To avoid an additional source of variability, an analyte easily detected by UV was selected. However, because UV is a non-selective detection technique, the co-migration of the injection standard with interfering peaks had to be excluded. Procaine (PROC), a compound that migrated as a cation before the EOF in the analytical conditions, was selected. PROC was added in both IS and unknown samples at a concentration of 50 μg mL^{-1}; online UV detection was performed at 195 nm for both PROC peaks at an effective length of 22 cm (Fig. 2A). A BGE plug was injected between both injections to insure sufficient resolution at 22 cm between all peaks, namely PROC (i.e., the cationic compound migrating before the EOF, peaks c and d), excipients (i.e., metacresol and glycerol, the neutral compounds detected in the EOF, peaks *), and INS (i.e., the anionic compound migrating after the EOF, peaks e and f). With a BGE plug corresponding to approximately 9% of the capillary length between both injections, the PROC peaks were sufficiently resolved to be easily integrated. Each TOF peak area of INS (IS and unknown samples, corresponding to peaks b and a in Fig. 2B) was corrected by its respective UV PROC peak (peaks d and c in Fig. 2A) for quantitative purposes. Finally, the ratio of the corrected area of the unknown sample (b/d)

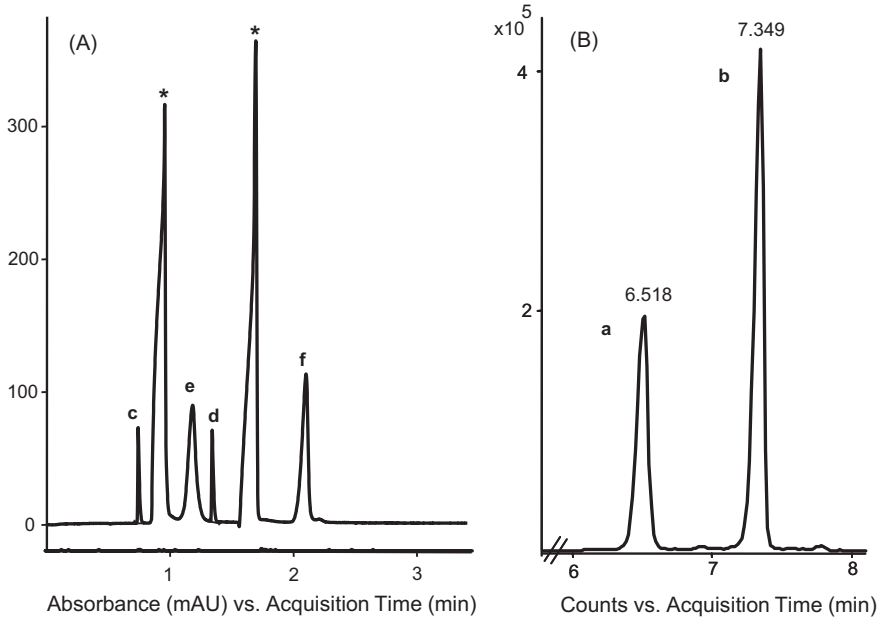

Fig. 2. :Electropherograms of Actrapid® obtained with the multiple injection approach: (A) UV detection and (B) ESI-TOF/MS detection. (a) IS (INS at 100 μg mL^{-1} from the first injection), (b) unknown sample (INS at around 175 μg mL^{-1} from the second injection), (c) PROC at 50 μg mL^{-1} from the first injection, (d) PROC at 50 μg mL^{-1} from the second injection, (e) IS (INS at 100 μg mL^{-1} from the first injection), and (f) unknown sample (INS at 175 μg mL^{-1} from the second injection). *Neutral excipients migrating with EOF.

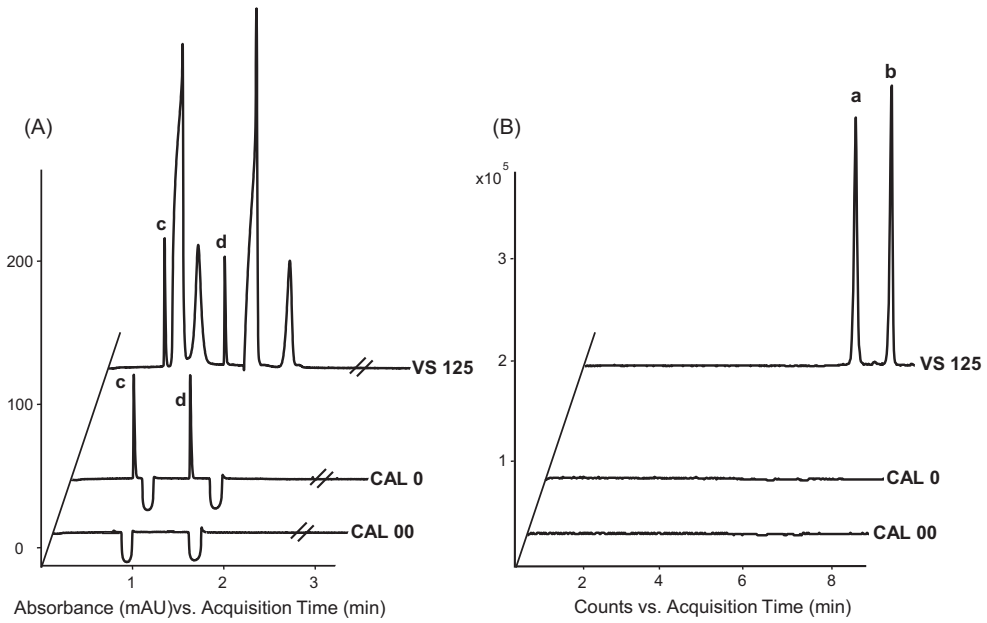

Fig. 3. Electropherograms obtained with the multiple injection approach: (A) UV detection and (B) ESI-TOF/MS detection. Injection of: water (CAL 00), water spiked with PROC at 50 μg mL^{-1} (CAL 0), and VS at 125%. (a) IS (INS at 100 μg mL^{-1} from the first injection), (b) unknown sample (INS at 175 μg mL^{-1} from the second injection), (c) PROC at 50 μg mL^{-1} from the first injection, and (d) PROC at 50 μg mL^{-1} from the second injection.

over the corrected area of IS (a/c) was calculated. The corresponding equation was (b/d)/(a/c). With this optimized procedure, the overall variability of the corrected areas was greatly improved (RSD ≤ 2%, $N = 5$).

3.2. Method validation

The quantitative performance of the CE-ESI-TOF/MS method was estimated on three separate series ($j = 3$). According to the SFSTP 2003 recommendations [33], as well as the identification of linear response function without significant intercept (Student's t-test, $\alpha = 0.05$) during the pre-validation process, validation protocol V1 was selected. Protocol V1 recommended a calibration out of matrix using two CS at the same concentration level (for example: at the target concentration or at a slightly higher concentration) and involved three concentration levels ($k = 3$) with two repetitions ($n = 2$) for VS, which was injected as the second injection sample ("unknown"). CS was set at a concentration corresponding at 100% of the formulation's concentration and was injected as the first injection sample ("IS"). The double role of the first injection (CS and IS) improved the throughput of the validation process, resulting in fewer injections. The trueness and precision were estimated for each concentration level. The former was expressed as relative bias while for the latter, the variances of repeatability and intermediate precision were computed from the estimated concentrations; the precision was expressed by RSD, as described in Rozet et al. [34]. The accuracy profile was then obtained according to the SFSTP 2003 recommendations ($\beta = 80\%$). The upper and lower tolerance limits represented the total error of the method, based on the tolerance interval.

3.2.1. Selectivity

The method selectivity was performed by comparing typical electropherograms obtained by injecting water (CAL 00), water spiked with PROC at 50 μg mL^{-1} (CAL 0), and a VS at 125%. As illustrated in Fig. 3A and B, no interference was observed at the migration time (MT) corresponding to the PROC and INS peaks in UV or ESI-TOF/MS measurements.

3.2.2. Trueness and precision

The results for trueness were expressed as relative bias (%) and assessed from the VS. As described in Table 1, the trueness was acceptable for the field of pharmaceutical formulation analysis since all values were lower than ±1.6%.

The precision was estimated by calculating the repeatability and intermediate precision at each concentration level of the VS. The RSD values presented in Table 1 were also acceptable for the field of pharmaceutical formulation analysis; they were in the range of 0.4–1.2% for repeatability, and between 0.8% and 1.6% for intermediate precision.

3.2.3. Accuracy

Accuracy takes into account the total error of the method and includes the combination of systematic (trueness) and random (precision) errors. The accuracy profile was selected as the decision tool to evaluate the method's capacity to quantify samples over the expected concentration range (75–125%). The accuracy profile for INS is illustrated in Fig. 4. As shown in Table 1, the upper and lower tolerance limits of the relative bias (%) were included into the acceptance limits (±5%). Consequently, the CE-UV-ESI-TOF/MS method could be considered accurate for INS over the investigated concentration range (131–219 μg mL^{-1}).

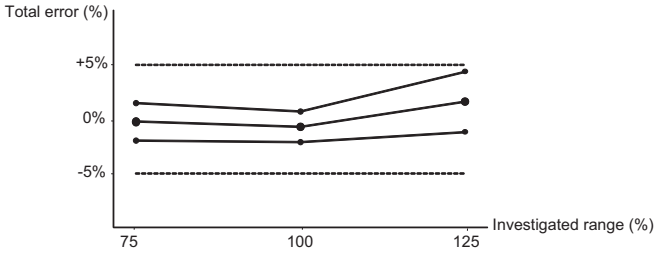

Fig. 4. Accuracy profile of INS in Actrapid® formulation obtained with the multiple injection approach ($j = 3$, $k = 3$, $n = 2$).

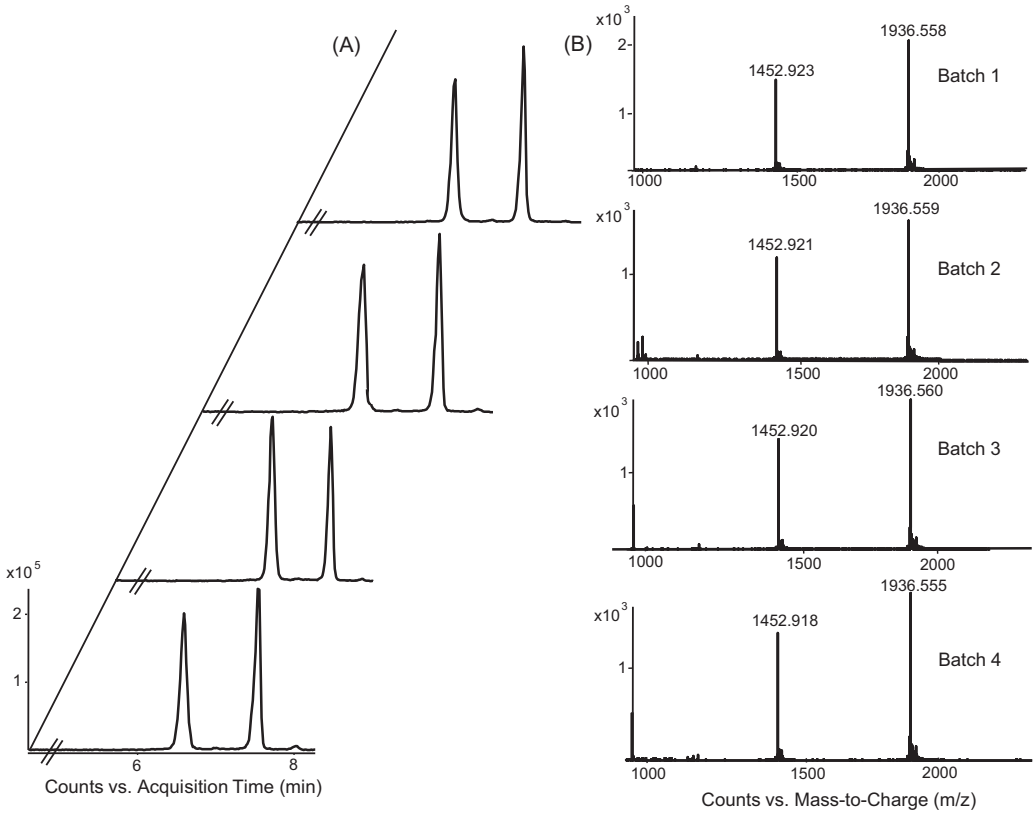

Fig. 5. CE-ESI-TOF/MS electropherograms and mass spectra of different samples of Actrapid® obtained with the multiple injection approach: (A) extracted ion currents (EIC) and (B) extracted mass spectra.

3.3. Application to real cases

To demonstrate the applicability of the CE-UV-ESI-TOF/MS method to real samples, the identification and quantitation of INS was achieved on pharmaceutical formulations of INS. Three samples were received from the Geneva Hospital Pharmacy (batches 1, 2, and 3, shipped from Switzerland), and one was purchased from the web (batch 4, shipped from Greece).

The unknown concentrations of INS samples, injected as second injection, were calculated with reference to CS at 100%, which was injected as the first injection. Two independent samples of each batch were prepared ($N = 2$), using the previously described methodology (20-fold dilution and addition of PROC as injection standard).

Table 1
Validation results for INS in Actrapid® formulation obtained with the multiple injection approach ($j = 3$, $k = 3$, $n = 2$).

Validation criterion	INS
Trueness	
Relative bias (%)	
131 μg mL^{-1} (75%)	−0.3
175 μg mL^{-1} (100%)	−0.7
219 μg mL^{-1} (125%)	+1.6
Precision	
Repeatability/intermediate precision (RSD, %)	
131 μg mL^{-1} (75%)	0.7/1.0
175 μg mL^{-1} (100%)	0.4/0.8
219 μg mL^{-1} (125%)	1.2/1.6
Accuracy	
Lower and upper tolerance limits of the total error (%)	
131 μg mL^{-1} (75%)	[−2.0;1.5]
175 μg mL^{-1} (100%)	[−2.2;0.7]
219 μg mL^{-1} (125%)	[−1.2;4.4]

Fig. 5 shows the EIC and extracted mass spectra of the four INS samples. The identity was confirmed by the m/z of both major extracted ions, owing to the mass accuracy determination afforded by the TOF analyzer. Concentrations of 99.9 ± 2.2%, 98.2 ± 2.2%, and 101.2 ± 2.2% (relative concentration to the reference batch ± confidence interval [%]) were calculated for the three batches obtained from the Pharmacy of the Geneva Hospital. A concentration of 100.2 ± 2.2% was calculated for the sample purchased on the web. All batches were complied with the expected specifications as their identities were confirmed and their concentrations fell within the expected limits (±5% around the target value).

4. Conclusions

A CE-UV-ESI-TOF/MS method was developed for the rapid identification and quantitation of pharmaceutical formulations containing intact proteins, such as insulin (INS). Our attention was first focused on estimating and preventing adsorption of the protein onto the capillary wall. The optimal CE conditions were obtained at basic pH values with the addition of acetonitrile to enhance CE performance and decrease adsorption. To improve CE-ESI-TOF/MS quantitation, a multiple injection approach and UV detection were chosen for correcting both ionization and injection variabilities. A fully validated strategy based on the accuracy profile was selected to demonstrate the ability of the CE-UV-ESI-TOF/MS method to quantify INS in Actrapid® formulations within a ±5% acceptance range. Four batches of INS formulation were successfully identified and quantified by the CE-UV-ESI-TOF/MS procedure. This strategy could be implemented in the field of quality control, as well as in the detection of counterfeits.

Conflict of interest

The authors have declared no conflict of interest.

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