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Comparison of liquid chromatography and capillary electrophoresis methods for quantification of sodium residuals

W. Briône [a], C. Herbots [b], C. Köttgen [a], S. Loix [a], M. Gibella [a], M. Bertrand [c], A. Ceccato [a,*]

[a] *Lilly Development Centre, Analytical Sciences R&D, Rue Granbonpré 11, B-1348 Mont-Saint-Guibert, Belgium*
[b] *Lilly Development Centre, Pharmaceutical Sciences R&D, Rue Granbonpré 11, B-1348 Mont-Saint-Guibert, Belgium*
[c] *Lilly Development Centre, Chemical Product R&D, Rue Granbonpré 11, B-1348 Mont-Saint-Guibert, Belgium*

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Abstract

Liquid chromatography (LC) and capillary electrophoresis (CE) methods were developed to perform the determination of residual sodium in mother liquors and successive washes of an active pharmaceutical ingredient (API). The addition of sodium chloride to the product solution results in rapid and complete crystallization of the API. The LC method was coupled to evaporative light scattering detection (ELSD) while the CE approach was based on indirect UV detection. Both methods were fully validated. Selectivity, response function, trueness, precision, accuracy, linearity and limits of detection (LOD) and quantification (LOQ) were the criteria investigated. The LC–ELSD method was found to be more sensitive than the CE/indirect UV approach. The methods were found to be valid over concentration ranges of 62–500 and 235–1500 ppm for the LC and the CE methods, respectively. Both methods were compared and used for the determination of actual samples coming from different batches of the same API chemical synthesis.

Keywords: Sodium; Validation; Capillary electrophoresis; Evaporating light scattering detector; Accuracy profiles

1. Introduction

The precise determination of inorganic ions is of prime importance in several disciplines such as clinical, environmental or analytical chemistry. The analysis of these anions and cations in aqueous samples can be made by several techniques such as atomic absorption spectrometry (AAS) [1–3], inductively coupled plasma–atomic emission spectrometry (ICP–AES) [4,5], flame photometry [4] or ion selective electrode methods [5–8]. While useful, these analytical methods are capable of analysing only a single analyte at a time. Some separation methods such as capillary electrophoresis (CE) or ion chromatography (IC) have been introduced as alternative approaches to these classical and sometimes time-consuming analytical methods. Currently, CE and IC techniques are probably the most used techniques in the field of analysis of inorganic ions.

Capillary electrophoresis (CE) is a powerful technique that has become popular as a standard analytical tool for the analysis of inorganic ions [9–18], essentially due to its high separation efficiency, its high speed of analysis and its relatively simple instrumentation. Even though some other detection modes such as fluorescence and conductivity were reported [15–18], the indirect UV method still remains the most useful approach for the determination of non-absorbing ions when CE instruments are applied [9–14].

The CE approach presented here is based on the utilization of a buffer system that dynamically coats the inner wall of fused silica capillaries with a double layer in order to obtain fast and reproducible analysis. The principle of the commercial reagent CEofix Cations HR kit is as follows: a buffer called "Initiator" containing a polycation (such as polybrene) is flushed through the capillary and is adsorbed to the wall surface. A second buffer called "Accelerator" containing a polyanion (such as poly(vinylsulfonate)) is then flushed. The polyanion adheres to the first layer of polycation, forming a double layer and restoring a strong electro-osmotic flow towards the cathode to allow the detection of cations [19–22]. This dynamic double coating approach is used to obtain better reproducibility.

Besides CE, ion chromatography (IC) and ion-exchange LC are probably the most popular analytical techniques for cation

* Corresponding author. Tel.: +32 10 47 63 10; fax: +32 10 47 63 86.
E-mail address: ceccato_attilio@lilly.com (A. Ceccato).

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analysis [23–30]. Traditionally, the analysis of inorganic ions has been performed on systems which employ conductivity or ELSD detection. The latter was reported for the determination of sodium in an active pharmaceutical ingredient (API) [28,29].

In the present work, the method developed is based on the utilization of a monolithic silica column used in the hydrophilic interaction chromatography (HILIC) mode with evaporative light scattering detection (ELSD). The HILIC mode employs polar stationary phases with mixed aqueous/organic mobile phases creating a stagnant enriched water layer around the polar stationary phase. This enriched layer allows analytes to partition between the two phases based on their polarity. In contrast to reverse-phase (RP) chromatography, where a non-polar stationary phase is employed and analyte elution is facilitated by the organic strength of the mobile phase, analyte elution is facilitated by the aqueous component of the mobile phase in HILIC mode. Although the HILIC mode is similar to the normal phase (NP) or polar organic mode, it is different in that the HILIC mobile phases contain a relatively high amount of water (5–40%), which can provide a significant solubility advantage for very hydrophilic samples [30].

As previously mentioned, one of the main problems for the analysis of inorganic ions in LC is the selection of an adequate detection mode. ELSD has been regularly reported as the detection mode of choice for the analysis of cations [23,28–30].

The aim of this paper is to report and to compare two different analytical methods dedicated to the analysis of residual sodium in mother liquors and aqueous washes of an API. The origin of sodium is explained as follows: an extractive work-up leads to the isolation of a solution of the API as a dihydrogenophosphate salt in water. As the hydrochloride salt of this API is much less soluble than the dihydrogenophosphate, adding chloride, as a NaCl solution, causes a rapid and complete crystallization of the API, with very minimal product losses in the mother liquor. The obtained crystals are then rinsed thoroughly to remove sodium phosphate, excess sodium chloride and excess phosphoric acid. This washing step is essential since the mother liquor is rich and residual salts may affect the API quality attributes such as hygroscopicity. The determination of sodium is therefore a useful indication of the quality of the washing step. Both the CE and LC methods were evaluated according to the validation strategy proposed by Hubert et al. [31,32], using accuracy profiles to select the most suitable calibration model [31,32]. The method selectivity and the assessment of precision, trueness and accuracy [31,33] at different concentration levels and in the determination of the limits of quantitation and the method linearity were also performed [31,34]. Finally, based on the validation results, both CE and LC methods were compared in regards to the intended use of the methods.

2. Experimental

2.1. Chemicals

The Initiator and Accelerator buffers were provided by Analis (Namur, Belgium) as a CEOfix Cations kit. The Cation HR Initiator buffer consists of a solution containing a polycation, 20 mM malic acid and 4-aminopyridine, adjusted at pH 4.2. The Cation HR Accelerator buffer consists of a solution containing a polyanion, 20 mM malic acid and 4-aminopyridine, adjusted to pH 4.2.

The separating buffer was a mixture of 20 mM malic acid, 4-aminopyridine and 18-crown ether (pH 4.3). Cation HR conditioner (LiOH 0.1 M) was used to clean the capillary between the injections. Both were purchased from Analis.

Ammonium acetate, used as an internal standard in the CE method, and acetic acid were both of p.a. quality from Merck (Darmstadt, Germany). Sodium chloride was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC supragradient from JT Baker (Deventer, The Netherlands) was used as organic modifier in the LC–ELSD method.

An uncoated fused silica capillary with a total length of 60.2 cm × 75 μm i.d. from Composite Metal Services Ltd. (West Yorkshire, UK) was used in this study; the effective separation length is 50.0 cm (from capillary injection to the capillary detection window).

A Zic Hilic silica column (250 mm × 4.6 mm i.d., 5 μm) from SeQuant (Umea, Sweden) was used for the separation in liquid chromatography.

Ultra pure water was obtained using a Milli-Q academic A10 from Millipore (Billerica, Massachusetts, USA).

2.2. Apparatus

The experiments in capillary electrophoresis were performed on a P/ACE System MDQ equipped with a diode array detection system from Beckman Coulter (Fullerton, CA, USA). A P/ACE Station software package (32 Karat Version 5.0) was used to control the system.

The detection was carried out at 200 nm in the indirect mode using 4-aminopyridine as the UV-absorbing buffer co-ion.

The experiments on the HILIC column were performed on an Alliance Waters 2695 Separations Module HPLC System (Waters Corporation, Milford, MA, USA). Waters Empower software was used to control the system and to acquire the data.

An evaporative light scattering detector (ELSD) SEDEX LT-ELSD Model 75 from SEDERE Inc. (Lawrenceville, New Jersey, USA) was used.

A conductimeter CON 110 series (Eutech Instruments, Singapore) was used to determine conductivity of the different solutions.

e-Noval® software (Arlenda, Liège, Belgium) was used to determine the accuracy profiles and other validation criteria. New-Daily® software from Arlenda was used to determine routine calibration curve equations and to calculate sodium sample concentration from during routine analysis.

2.3. Separation techniques

2.3.1. Liquid chromatography

The liquid chromatography (LC) analysis on the HILIC column was performed in the isocratic mode using a mobile phase of 50 mM ammonium acetate adjusted to pH 4.5 and acetonitrile (25:75, v/v). The flow rate was 1.5 ml/min. The injection

volume was set to 20 μl. The detection was performed using an ELSD detector set to 60 °C with a 2.5 bar pressure. Gain of the apparatus was set to 6.

2.3.2. Capillary electrophoresis

The conditioning of the capillary was performed daily according to the following sequence: the capillary is rinsed for 1 min with the 0.1 M LiOH solution, 1 min with the Initiator buffer, 2 min with the Accelerator buffer, 0.5 min with the 0.1 M LiOH solution and finally for 0.5 min with ultra pure water. The same 20 psi pressure was applied for all the rinses.

Between injections, the capillary was successively rinsed with the Initiator buffer (0.5 min), the Accelerator buffer (0.5 min) and the separating buffer (1.5 min). After each completed analysis, the capillary was rinsed with the 0.1 M LiOH solution (0.5 min) and ultra pure water (0.5 min). The same 20 psi pressure was applied for all steps.

The injection was performed in the hydrodynamic mode, using a positive pressure of 0.5 psi for 5 s. The temperature was maintained at 25 °C and the detection was achieved at 200 nm. A 30 kV voltage was applied across the capillary for 5.5 min.

2.4. Standard solutions

2.4.1. Solutions used for method validation

For the LC–ELSD analytical method, a stock solution of sodium chloride was prepared by weighing an appropriate amount to reach a sodium concentration of 2000 ppm. This solution was then diluted to obtain solutions ranging from 10 to 500 ppm.

For the CE/indirect UV analytical method, a stock solution of ammonium acetate (IS) was prepared by weighing an appropriate amount to reach an ammonium concentration of 2000 ppm. Stock solutions were combined and diluted to obtain a fixed concentration of 150 ppm ammonium and sodium concentrations ranging from 100 to 1500 ppm.

2.4.2. Standard solutions for routine analysis

A 2000 ppm stock solution of sodium was prepared and diluted adequately to get three concentration levels of standard solutions.

For the LC–ELSD analytical method, the concentration levels were 50, 100 and 500 ppm. These standard solutions were prepared three times to get three independent standard solutions at each concentration level.

For the CE method, the concentration levels were 250, 750 and 1500 ppm in sodium. These standard preparations were prepared three times to get three standard solutions at each concentration levels. The final concentration of IS was fixed to 150 ppm of ammonium.

2.5. Sample preparation

Samples consisted of mother liquors and aqueous washes from four different batches of the same chemical synthesis of a new active pharmaceutical ingredient (API). These aqueous solutions required dilution if the sodium concentration was above the valid range. The right amount of IS was weighed directly into the flask to achieve the final concentration, fixed to 150 ppm of ammonium.

Both developed methods were used in routine analysis to measure the sodium amount in different samples coming from different batches of the same chemical synthesis.

3. Results and discussion

3.1. Selection of the CE conditions

The CEofix Cation HR Kit consists of different solutions such as the Cations HR initiator, the Cations HR Accelerator, the separating buffer and the Cations HR conditioner. 4-Aminopyridine (probe) is added to the buffers to allow the UV indirect detection. The displacement of the probe by the ion being analysed provides the basis of the detection and gives rise to negative peaks that are reversed by the software to get positive peaks [19,35,36]. Malic acid is used as a buffer and the 18-crown ether is added in the background electrolyte (BGE) to get a good separation between sodium and ammonium [24].

The CE method described under the method description section gave good results for the determination of sodium. The method was developed to allow determination in a wide concentration range.

3.2. Selection of the LC conditions

The LC method for the quantification of sodium was investigated. The first experiments were performed on the column described above. The injection volume was adapted and tested from 5 to 40 μl and gain of the ELSD detector from 9 to 6 to be in a right concentration range (i.e. to avoid saturated peak). The final method is described above and allows the quantification of sodium over a large concentration range.

3.3. Validation

3.3.1. Prevalidation step

The relationship between the response (i.e. the chromatographic signal) and the concentration (amount) of the analyte in the sample system is a very important parameter that must be considered in the validation of an analytical method [31,34–42]. Typically, the linear regression model is used to explain the response function of LC or CE methods, but this model is not always the most appropriate. By using the approach based on two-sided 95% β-expectation tolerance intervals [31,37,43] for total measurement error (including bias and precision), the most appropriate response function model can be selected by taking into account the performance of future individual assays and the ability to reduce the risk of rejecting in-study runs.

This validation approach consists in using two kinds of standards: the calibration standards are used to set up the calibration model while the validation standards are used to estimate the precision, trueness and accuracy of the method. In the present study, four series with three and six concentration levels were used for the calibration and the validation standards, respectively.

Three repetitions per level were performed. The concentration ranges investigated were 10–500 ppm for the LC method and 100–1500 ppm for the CE method. These validation experiments (preparation and analyses) were performed by three different operators.

Different regression models were evaluated: linear regression, linear regression after logarithm transformation, weighted linear regression, quadratic regression, weighted quadratic regression, linear regression after square root transformation, weighted linear regression after square root transformation, weighted linear regression after logarithm transformation, linear regression through 0 fitted using the highest level only (500 ppm for LC and 1500 ppm for CE) and linear regression through 0 fitted using the level 4 only (100 ppm for LC and 750 ppm for CE). The selection of the most suitable model was made using the accuracy profile [31,37]. The accuracy profile is used as a tool to decide the capability of the method to give results inside the acceptance limits. It is obtained by linking on one hand the lower bounds and on the other hand the upper bounds of the β-expectation tolerance limits calculated at each concentration level.

The β-expectation tolerance interval means that the method will be able to give a result within this interval 95 times out of 100 experiments. Therefore, the selection of the regression model can be done by considering the model for which the β-expectation tolerance interval stays within a pre-defined acceptance criterion.

Considering the objective of the present method, i.e. the determination of residual sodium and the concentration range investigated that is relatively low, it is reasonable to set the acceptance limit to 15%.

Among the different accuracy profiles obtained for the sodium determination, the linear regression and the quadratic regression models were selected for LC and CE methods, respectively, since they represent the most suitable regression models. Figs. 1 and 2 illustrate the accuracy profiles corresponding to the selected models for LC and CE methods. As is shown in Fig. 1, the linear regression model is not suitable for the lowest concentration but from the 75 ppm concentration level, it gives the guarantee that each further measurement of unknown samples will be included within the tolerance at the 5.0% level. Fig. 2 shows clearly that the quadratic regression model covers nearly all the concentration range investigated.

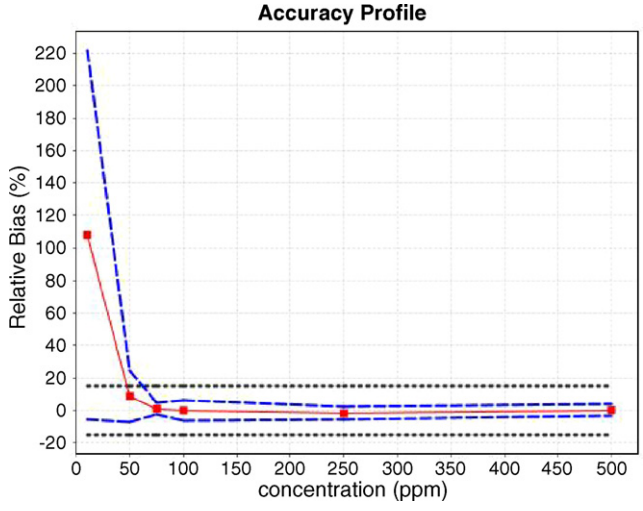

Fig. 1. Accuracy profiles of sodium determinations using LC/HILIC/ELSD analytical method obtained from enoval® using the linear regression model with three concentration levels (50, 100, 500 ppm).

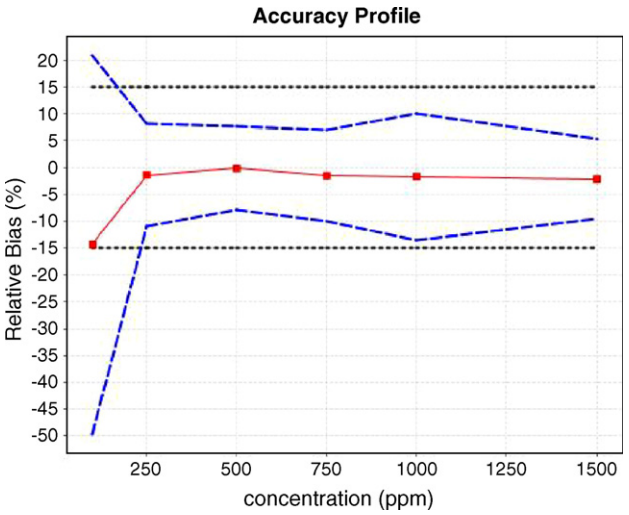

Fig. 2. Accuracy profiles of sodium determinations using CE/indirect UV analytical method obtained from enoval® using the quadratic regression model with three concentration levels (250, 750, 1500 ppm).

It is important to note that the objective of the methods was to allow the determination of sodium in a relative wide range of concentration since it was not possible to predict the sodium concentration. The main objective of the methods was to determine if the washing of the precipitate was complete.

3.3.2. Selectivity

The selectivity of the methods was investigated. Fig. 3 illustrates the separation of the API, sodium and chloride ions using the LC method while Fig. 4 illustrates the electropherogram obtained when a solution containing the API, the IS and 250 ppm of sodium was analysed using the CE method.

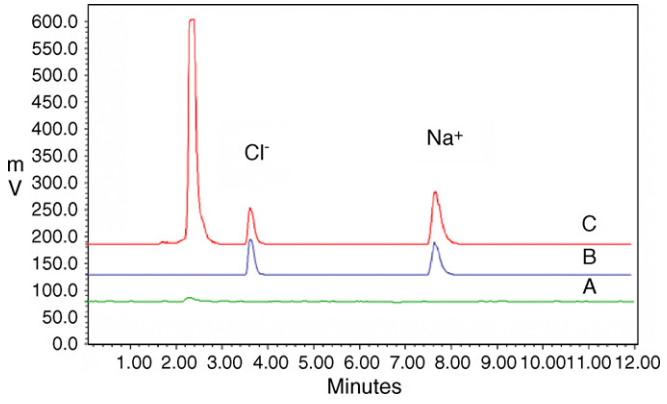

Fig. 3. Chromatogram illustrating the separation of sodium, chloride and API using the LC–ELSD method. (A) Blank solution; (B) standard at 100 ppm; (C) typical sample (see text for chromatographic conditions).

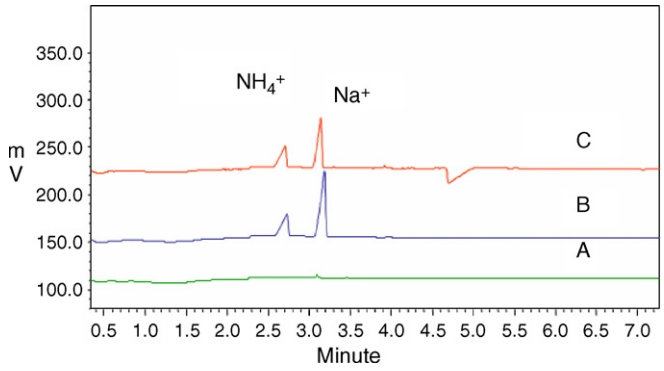

Fig. 4. CE Separation of sodium and ammonium in a solution containing the API. (A) Blank solution; (B) standard solution at 250 ppm Na^+; (C) typical sample (see text for conditions).

3.3.3. Response function

As previously mentioned under the pre-validation step, the linear regression was used for the LC method while a quadratic regression was used for the CE method. In both cases, four series ($k = 4$) with three concentrations levels and three repetitions at each level were performed. One equation was obtained for each series and the average equation was calculated (Table 1).

Table 1
Validation of the LC and the CE methods

Criterion	LC–ELSD	CE/indirect UV
Response function	Linear regression Slope = 2.10×10^4 Intercept = -3.24×10^5	Quadratic regression Slope = 7.51×10^{-3} Intercept = 0.14 Quadratic term = 1.44×10^{-6}
Conc. range	50–500 ppm	100–1500 ppm
Trueness (expressed as relative bias)	10 ppm: 108.00% 50 ppm: 8.86% 75 ppm: 1.09% 100 ppm: 0.10% 250 ppm: −1.65% 500 ppm: 0.31%	100 ppm: −14.4% 250 ppm: −1.44% 500 ppm: −0.17% 750 ppm: −1.58% 1000 ppm: −1.71% 1500 ppm: −2.17%
Repeatability (R.S.D., %)	10 ppm: 5.4% 50 ppm: 7.0% 75 ppm: 1.2% 100 ppm: 1.9% 250 ppm: 1.8% 500 ppm: 1.4%	100 ppm: 11.96% 250 ppm: 3.36% 500 ppm: 2.87% 750 ppm: 2.62% 1000 ppm: 2.04% 1500 ppm: 1.67%
Intermediate precision (R.S.D., %)	10 ppm: 32.7% 50 ppm: 7.0% 75 ppm: 1.5% 100 ppm: 2.5% 250 ppm: 1.8% 500 ppm: 1.4%	100 ppm: 14.41% 250 ppm: 3.94% 500 ppm: 3.25% 750 ppm: 3.38% 1000 ppm: 3.99% 1500 ppm: 2.69%
Linearity	Slope: 0.9900 Intercept: 4.28 R^2: 0.9988	Slope: 0.9830 Intercept: −1.76 R^2: 0.9970
LOD/LOQ	LOD: 30.2 ppm LLOQ: 62.2 ppm ULOQ: 500 ppm	LOD: 116.9 ppm LLOQ: 234.5 ppm ULOQ: 1500 ppm

3.3.4. Trueness

Trueness gives information on systematic error and is expressed in terms of relative bias (%). It refers to the closeness of agreement between the mean value obtained from a series of measurements and the conventionally accepted value or reference value [31]. In the present study, it was assessed using validation standards at six concentration levels, ranging from 10 to 500 ppm for the LC method and from 100 to 1500 ppm for the CE method ($k = 4$, $n = 6$). Three independent validation standard solutions were injected for each concentration level. As is shown in Table 1, the proposed methods can be considered as true since the bias did not exceed 15% except for the lowest concentration for the LC study. At this concentration level, the observed relative bias (higher than 100%) illustrates the importance of the systematic error.

3.3.5. Precision

The precision of an analytical method gives information on the random error. It expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions [31]. It was estimated by measuring repeatability and intermediate precision at different concentration levels over the concentration ranges studied. The variance of repeatability and intermediate precision as well as the corresponding relative standard deviation (R.S.D., %) were calculated from the estimated concentrations. Except for the lowest concentrations, the R.S.D. values presented in Table 1 were around 1–2% for the LC method and 2–3% for the CE method. Except for the lower concentration levels, the results illustrate the good precision of the proposed methods for sodium quantification.

3.3.6. Accuracy

Accuracy takes into account the total error, i.e. systematic and random errors, related to the test result. It expresses the closeness of agreement between the calculated value and the accepted reference value, namely the conventionally true value [31]. It is assessed from the accuracy profiles illustrated in Figs. 1 and 2. They show clearly that the LC method of determination of sodium is accurate between 75 and 500 ppm while the CE method is accurate between 250 and 1500 ppm. The upper and lower β-expectation tolerance limits expressed in ppm presented in Table 2 as a function of the known concentrations demonstrate that the methods are accurate within the 75–500 ppm range, and 250–1500 ppm, for LC and CE methods, respectively, since the limits of tolerance of the errors (relative β-expectation tolerance limits) do not exceed the acceptance limits (±15%). However, at the lower concentration levels, the accuracy of the methods is clearly not suited to their objective. Table 2 also indicates the significant risk of reporting a concentration value with an error higher than 15% at the lowest concentration level.

3.3.7. Linearity

Linearity is the ability for an analytical method to give results directly proportional to the concentrations (amounts) of ana-

Table 2
Accuracy of the method

Analytical method	Concentration (ppm)	β-Expectation limit (ppm)	Relative β-expectation limit (%)	Risk (%)
LC/HILIC/ELSD	10	[9.407, 32.19]	[−5.934, 221.9]	100.00
	50	[46.43, 62.43]	[−7.147, 24.86]	33.72
	75	[73.09, 78.54]	[−2.549, 4.721]	2.867×10^{-05}
	100	[93.92, 106.3]	[−6.077, 6.268]	5.786×10^{-03}
	250	[235.4, 256.4]	[−5.855, 2.561]	2.867×10^{-05}
	500	[485.3, 517.9]	[−2.948, 3.577]	2.867×10^{-05}
CE/indirect UV	100	[50.31, 121.0]	[−49.69, 20.98]	64.80
	250	[222.5, 270.3]	[−11.01, 8.130]	1.304
	500	[460.3, 538.0]	[−7.937, 7.606]	0.1144
	750	[674.2, 802.1]	[−10.11, 6.942]	0.6756
	1000	[864.8, 1101]	[−13.52, 10.11]	4.697
	1500	[1356, 1579]	[−9.612, 5.276]	0.3736

lyte in the sample within a defined concentration range [31]. This criterion has to be applied only to results (concentrations or amounts), not to responses (i.e. chromatographic signals). A regression line can therefore be fitted between the back-calculated concentrations versus the introduced concentrations applying the linear regression model based on the least squares method. The regression lines were calculated for both techniques and the equations are presented in Table 1. Graphic illustrations of linearity are presented in Fig. 5. They show clearly the linear relation between the back-calculated concentration and the actual concentration of sodium. The dashed limits correspond to the accuracy profile while the dotted line corresponds to the acceptance limits, set at 15% in the present example. This graph also illustrates the accuracy of the method, expressed in the concentration unit.

3.3.8. Detection and quantitation limits

The limit of detection is defined as the lowest amount of the considered substance that can be detected, but not necessarily quantified as an accurate value [31]. The limits of detection of the considered compounds in the present study were estimated using the mean intercept of the calibration model and the residual variance of the regression. The lower limit of quantitation (LLOQ) of an analytical procedure is defined as the smallest quantity of the considered substance in the sample that can be quantitatively determined under the experimental conditions with well-defined accuracy [31], i.e. taking into account the systematic and random errors [41,42]. This definition can also be applied to the upper limit of quantitation (ULOQ), which is therefore the highest concentration or quantity that can be determined with a well-defined accuracy. The limits of quantitation can therefore be obtained by calculating the smallest and highest concentration beyond which the accuracy limits or β-expectation tolerance limits go outside the acceptance limits. Limits of detection and quantitation for the both analytical methods are mentioned in Table 1. The concentration range for which the method is validated is comprised between the lower and the upper limits of quantitation. Clearly, the LOD and LOQ values achieved for the LC method are lower than those obtained for the CE approach, indicating that the latter is less sensitive.

3.4. Routine analysis

Both LC and CE methods developed were applied to the analysis of different samples of mother liquors and washes

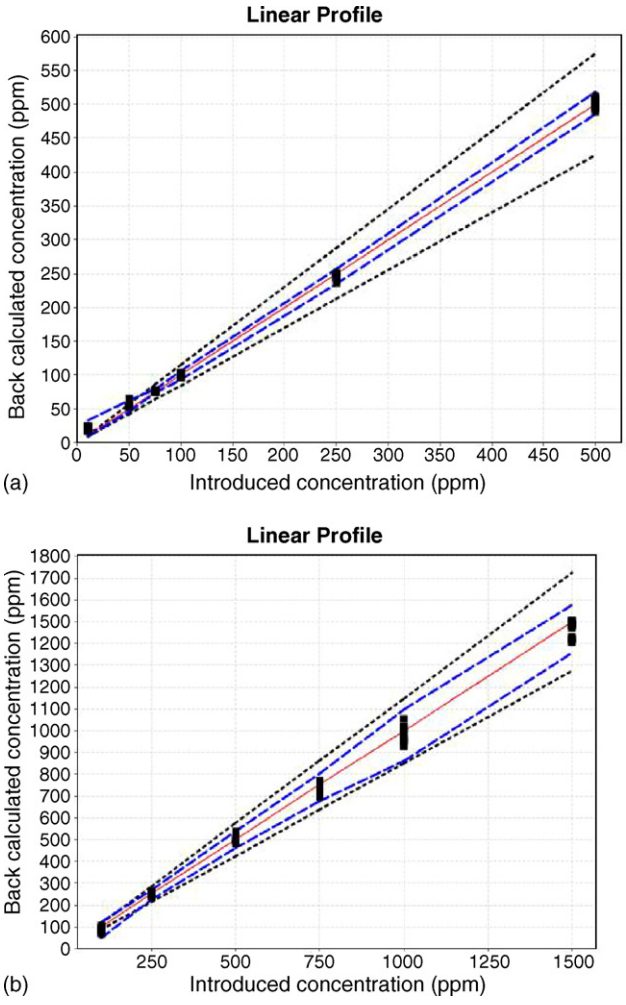

Fig. 5. Linear profile of sodium using (a) LC–ELSD and (b) CE/indirect UV methods. The dashed limits correspond to the accuracy profile, i.e. the β-expectation tolerance limits expressed in the concentration unit (ppm). The dotted curves represent the acceptance limits at 15% expressed in the same concentration unit.

Table 3
Samples analytical results

Process	Sample	LC–ELSD mean ± S.D. (ppm) ($n = 3$)	CE/indirect UV mean ± S.D. (ppm) ($n = 3$)	Conductivity (μS/cm)
A	Mother liquor "A"	3220 ± 85	3140 ± 72	11900
	First wash "A"	166 ± 11	<LOD	1465
	Second wash "A"	<LOQ	<LOD	536
	Third wash "A"	<LOQ	<LOD	440
B	Mother liquor "B"	2908 ± 23	2687 ± 72	9500
	First wash "B"	2879 ± 94	2798 ± 59	10010
	Second wash "B"	194 ± 8	<LOD	1524
	Third wash "B"	<LOQ	<LOD	866
	Fourth wash "B"	<LOQ	<LOD	489
	Sixth wash "B"	<LOD	<LOD	395
C	Mother liquor "C"	5681 ± 222	5503 ± 69	17080
	First wash "C"	3249 ± 42	3076 ± 98	11080
	Second wash "C"	252 ± 3	<LOQ	1903
D	Mother liquor "D"	6306 ± 101	6483 ± 157	18370
	First wash "D"	744 ± 15	735 ± 11	3870
	Second wash "D"	<LOQ	<LOD	689
	Third wash "D"	<LOQ	<LOD	510

corresponding to the chemical synthesis of a new API. Each sample was analysed independently three times. Calibration curves for sodium quantification were linear regression and quadratic regression models for LC and CE, respectively, as demonstrated during the validation. Moreover, the conductivity of the different samples tested was performed in order to investigate the relation between the sodium concentration and the conductivity.

As shown by the results presented in Table 3, the LC–ELSD method is more sensitive than the CE method.

However, it is interesting to note that for the higher concentrations, the determination of sodium of the same samples using either the LC–ELSD or CE methods gave similar results, meaning that both methods can be used confidently. It is also interesting to note that all results obtained were well correlated with the conductivity measurement (Table 3). This indicates that monitoring conductivity during the process can provide a useful information about the efficiency of the washing step. In the present study, the LC–ELSD method seems to be the most useful analytical method since it was possible to quantify, with precision, trueness and accuracy very low amounts of sodium.

4. Conclusions

The determination of sodium was performed using two different analytical methods. The first method developed was a CE method coupled to indirect UV detection while the second technique utilized a LC separation on a HILIC stationary phase coupled to ELSD detection. Both methods were validated according to the accuracy profile determination approach described in previous studies and were found to meet the requirements for the determination of sodium. The developed methods were compared and were both successfully used to analyse actual samples coming from the chemical synthesis process of a new active pharmaceutical ingredient. The LC–ELSD method was found to be more sensitive than the CE approach although both techniques gave confident results. The LOD of the LC–ELSD method was approximately 30 ppm compared to 120 ppm for the CE approach. Results obtained from the analysis of actual samples were correlated with conductivity measurements, indicating that the measurement of conductivity may be used as a process-monitoring tool.

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