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COMPARISON OF COLUMN-COUPLED ELECTROPHORESIS WITH LIQUID CHROMATOGRAPHY METHODS IN FOOD ANALYSIS OF QUININE

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Comparison of column-coupled electrophoresis with liquid chromatography methods in food analysis of quinine (QUI) is presented in this work. The capillary isotachophoresis (CITP) on-line coupled with capillary zone electrophoresis (CZE) and hyphenated with fibre-based spectrophotometric diode array detection (DAD) was compared with, (i) high performance liquid chromatography (HPLC) method with DAD detection, and (ii) HPLC method with fluorescence detection (FD). These methods were compared through their performance parameters and determined concentrations of QUI in beverages. The concentrations of QUI in two selected bitter drinks determined by the CITP-CZE-DAD method were in a good accordance with the HPLC-DAD and HPLC-FD methods. In addition, the electrophoretic method, as well as the chromatographic methods, was able to separate potential QUI related impurities from the QUI peak. The CITP-CZE-DAD method provided excellent performance parameters that were comparable (precision, accuracy, LOD, robustness) or even better (separation efficiency) than those ones provided by the chromatographic methods. Moreover, the effectivity of the electrophoresis method was higher when considering cost of analysis (equipment, consumption of separation systems), environmental aspects (organic *vs*. aqueous solvents), on-line sample pretreatment (CITP preconcentration and sample clean-up suitable also for the more complex matrices). Considering these findings, CITP-CZE-DAD was approved as a routine automatized method for the highly reliable quality food control.

Keywords: column coupled electrophoresis – food analysis – quinine – high performance liquid chromatography – detection – validation

INTRODUCTION

Quinine (QUI) is a naturally fluorescing, crystalline alkaloid extracted from the bark of the cinchona tree having antimalarial, analgesic, antipyretic, antiseptic and muscle-relaxant properties. It is used in medicine to treat malaria and to treat or prevent

nighttime leg cramps. The resistance of the parasite *Plasmodium falciparum* to QUI appears to be significantly less than with chloroquine, thus QUI is still widely used (McCalley, 2002). QUI is also used as a flavouring agent to provide the bitter taste in beverages. The levels of QUI in bitter drinks are regulated by health authorities and can be added up to a level of 80-100 mg/kg, see e.g. refs. (U.S. Department of Health and Human Services, 2010; Lester, 1994).
High performance separation techniques such a HPLC and CE (CZE, MEKC, CITP) and a variety of detection systems (UV, DAD, LIF, MS) have been reported in the literature for the analysis of QUI in model as well as real (beverages, pharmaceuticals, urine, plasma) matrices (Altria & Simpson, 1988; Buchberger et al., 2010; Feas et al., 2009; Hodel et al., 2009; Horie et al., 2006; Ibrahim et al., 2007; Junior et al., 2009; Marini et al., 2010; Quing & Jing, 2001; Reijenga et al., 1985; Samanidou et al., 2004; Steiner & Hassel, 2005; Tsimachidis et al., 2008; Zaugg & Thorman, 2001; Zhang et al. 2007). From among those, the methods in Table 1 have been used for the analysis of QUI in beverages.

Table 1. Methods used in analysis of QUI in beverages

Method	**Detection**	**LOD**	**Ref.**
HPLC	UV FD	20 ng/ml 4 ng/ml	Quing & Jing, 2001
HPLC	UV	2000 ng/ml	Horie et al., 2006
HPLC	FD	300 ng/ml	Samanidou et al., 2004
CITP	UV	5000 ng/ml	Reijenga et al., 1985
CZE	UV	180 ng/ml	Tsimachidis et al., 2008

Although HPLC remains the most common method for the analysis of QUI, there has been a significant growth of interest in capillary electrophoresis (CE) in the last few years. Moreover, on-line combination of capillary isotachophoresis (CITP) with capillary zone electrophoresis (CZE) considerably reduce, (i) the concentration limits of detection (cLOD) ,and (ii) external sample manipulation when compared to current (single column) CZE, and enhance (iii) separation selectivity, (iv) real applicability.
In the present work, several analytical separation methods with different types of detection were used for the determination of QUI in bitter drinks. The principal goal was to provide a comparison of the CITP-CZE-DAD method developed in our laboratory with the high performance liquid chromatography separation methods, such HPLC-DAD and HPLC-FD in order to illustrate potentialities (benefits and limitations) of the particular methods in the quality food control. Here, the separation techniques used offer different separation mechanisms (electrophoretic, chromatographic) for the separation of the sample constituents, while spectrophotometric DAD can be effectively utilized for a control of purity of the analyte zone when analyzing the samples with variable matrix composition (commercial beverages), thus showing the quality of the separation process. FD similarly to DAD can indicate QUI related impurities with a relatively high probability because of a highly selective detection response (DAD spectrum, fluorescence).

EXPERIMENTAL DETAILS

Instrumentation CITP-CZE

A capillary electrophoresis analyser EA-102 (Villa-Labeco, Spišská Nová Ves, Slovakia), assembled in the column-coupling configuration of the separation unit, was used in this work for performing the CITP-CZE runs. Electrode compartments with hydrodynamically (membrane) closed connecting channels to the separation compartment (Villa-Labeco) were employed. The samples were injected by a 30 µl internal sample loop of the injection valve of the analyzer. A CITP column was provided with an 800 µm i.d. fused silica capillary tube (Polymicro Technologies, Phoenix, USA) of a 90 mm length and a contactless conductivity detector. A CZE column (Polymicro Technologies) was the same as the CITP one except for a 300 µm i.d. and a 140 mm length.

A multiwavelength spectrophotometric absorbance diode array detector Smartline PDA Detector 2800 (Knauer, Germany) was connected to an on-column photometric detection cell, mounted on the CZE column, via optical fibres. The detector operated under the following conditions: (1) scanned wavelength range 200-600 nm; (2) integration time 6 ms; (3) scan interval 0.2 s; (4) number of accumulations 1. The spectrophotometric detector was set at a 330 nm detection wavelength when evaluating the performance parameters. The migration and spectral data were acquired and processed by a EuroChrom program (version 3.05, Knauer).

Instrumentation HPLC-DAD

The chromatographic apparatus consisted of a LC Agilent Infinity System equipped with an Infinity 1260 gradient pump, a 1260 HiPals automatic injector, a column thermostat 1290, a photo-diode array detector Infinity 1290 and a computer with Chemstation software for data registration and calibration (Agilent Technologies, USA). The chromatographic column was a Poroshell RP C18, 3,0mm x 50mm i.d., 2.7 µm particle size (Agilent Technologies, USA) thermostated at 30°C. The detection wavelength was 330 nm and the injection volume was 10 µl.

Instrumentation HPLC-FLD

The liquid chromatography system HP 1050 Series was equipped with a fluorescence detector Agilent 1100 Series and a computer with Chemstation software for data registration and calibration (Agilent Technologies, Waldbron, Germany). The excitation and emission wavelengths of the detector were set at 325 nm and 375 nm respectively. The injection volume was 20 µl. Separations were done on a chromatographic column Luna 5u C18(2), 150 x 4.6 mm i.d., 5µm particle size (Phenomenex, Germany). The column was kept at room temperature.

Instrumentation for fluorimetry

Spekol 11 (Carl Zeiss, Jena, Germany) with the silica fluorimetric cuvette of a 10 mm inner width was employed for the comparative fluorimetric method (Havránek, 1998). The excitation wavelength of 370 nm and 0.1 mol/l H_2SO_4 comparative solution were used for the determination of QUI.

Data evaluation and performance parameters

Performance parameters of the method were evaluated according to the ICH guideline (ICH Harmonised Tripartite Guideline, 2005). Peak area of QUI from the separation methods was corrected for the migration time (Huang, 1989). Parameters of calibration line for QUI were calculated by using QCExpert ver.2.5 statistical software (Trilobyte, Prague, Czech Republic).

Chemicals and samples

The electrolyte solutions were prepared from chemicals obtained from Merck (Darmstadt, Germany), Sigma-Aldrich (Steinheim, Germany), and Fluka (Buchs, Switzerland) in water demineralised by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona, U.S.A.). All chemicals used were of analytical grade. The solutions of the electrolytes were filtered before use through disposable membrane filters of a 0.8 μm pore size (Millipore, Molsheim, France). Methanol (LC-MS grade) was purchased from J.T.Baker (Deventer, Netherlands). Ammonium acetate was purchased from Fluka (Chemika, Switzerland). Acetonitrile (HPLC grade) and ammonium formate were obtained from Sigma-Aldrich.

Quinine hydrochloride dihydrate (QUI) was obtained as a reference substance from Sigma. Beverages (bitter drinks), namely Kinley, Schweppes, were obtained from the local stores. No declared content was available for Kinley and Schweppes; however, the levels of QUI in bitter drinks are regulated by USA health authorities and can be added up to a level of 80 mg/kg (Code of Federal Regulations, 2010).

Procedures for sample and standard solution preparations

For CITP-CZE experiments, the stock solution of QUI reference substance (i.e. standard stock solution) was prepared by dissolving 10 mg of the powder in 10 ml of demineralised water with 0.5 mmol/l H_2SO_4 and 1 mmol/l NaH_2PO_4. Working standard solutions were made by an appropriate dilution of the standard stock solution with the mixture consisted of 0.5 mmol/l H_2SO_4 and 1 mmol/l NaH_2PO_4 in demineralised water. Individual concentrations of working standard solutions for calibration curve were 10; 30; 75; 100; 150 ng/ml. For the evaluation of recovery, three different concentrations were used 15; 60; 110 ng/ml. Pure as well as spiked beverage solutions were also made in 0.5 mmol/l H_2SO_4 and 1 mmol/l NaH_2PO_4 background and after an appropriate dilution they were directly injected.

For HPLC-DAD, the stock solution of QUI reference substance (i.e. standard stock solution) was prepared by dissolving 47.72 mg of the powder in 50 ml of demineralised water. The concentration of the stock solution was 0.78 mg/ml. The stock solution was appropriately diluted with 5 mmol/l ammonium formate in order to obtain working standard solutions for calibration curve with following concentrations: 15.6; 39; 78; 156; 390; 780 ng/ml. Other three working solutions with respective concentrations 50; 250; 500 ng/ml were prepared for the evaluation of recovery.

For HPLC-FD, the stock solution of QUI reference substance (i.e. standard stock solution) was prepared by dissolving 44.5 mg of the powder in 25 ml of demineralised water. The concentration of the stock solution was 1.78 mg/ml. Working standard solutions of concentrations 1.78; 17.8; 178 and 1780 ng/ml for calibration curve were made by an appropriate dilution of the standard stock solution in demineralised water. For the evaluation of recovery, three working solutions of concentrations 8.9; 44.5; 890 ng/ml were prepared in demineralised water.

The solutions and samples for the comparative fluorimetric method (Havránek, 1998) were prepared in demineralised water. The concentration of the standard stock solution of QUI was 0.1 mg/ml, the concentration of the stock solution of H_2SO_4 was 0.1 mol/l. These two solutions were combined in 25 ml volumetric flasks in order to prepare 4; 8; 12; 16; 20 μg/ml calibration solutions of QUI. The beverages were degassed by the sonication (10 min), then appropriately diluted by the stock solution of H_2SO_4 and measured.

RESULTS AND DISCUSSION

Separation conditions

CITP-CZE method. The buffer constituents (sodium acetate, acetic acid, beta-alanine - BALA, NaH_2PO_4), concentration of leading and carrier cations (leading cation: 10-20 mmol/l, carrier cation: 20-50 mmol/l), pH (4-6), and driving currents (CITP: 100-500 μA, CZE: 50-200 μA) in the CITP-CZE separations were tested in order to find an experimental optimum considering (i) rapid analysis, (ii) minimization of thermal, adsorption and electromigration dispersion effects, (iii) sufficient resolution of the analyte, and (iv) good compatibility of the on-line coupled separation systems (Mikuš, 2011). Prior to the use, the capillaries were not treated by any rinsing procedures to suppress an electroosmotic flow (EOF). A dynamic coating of the capillary wall by means of methyl hydroxyethyl cellulose (MHEC 30 000; Serva, Heidelberg, Germany) present in leading and background electrolyte solutions served for this purpose. The separating electrolytes in the capillaries were replaced by the fresh ones between each run. CITP and CZE analyses were carried out in the cationic regime of the separation (i.e. cathodic movement of the analytes) with direct injections of the samples. The experiments were performed in constant current mode at 20°C. The driving currents applied were 300 μA (CITP) and 120 μA (CZE). The optimized electrolyte systems, considering all above mentioned aspects, are the following: (i) CITP electrolytes, leading electrolyte (LE): 10 mmol/l sodium acetate, 20 mmol/l acetic acid, 1 mmol/l NaH_2PO_4, 0.1% (w/v) MHEC, pH 4.5; terminating electrolyte (TE): 10 mmol/l BALA, 10 mmol/l acetic acid, pH 4.3; (ii) CZE carrier electrolyte: 25 mmol/l BALA, 25 mmol/l acetic acid, 0.1% (w/v) MHEC, pH 4.3.

HPLC-DAD method. Gradient elution of the analyte was performed using two mobile phases as shown in table 2. Mobile phase A consisted of 5 mmol/l ammonium formate, and mobile phase B consisted methanol. The flow rate of the mobile phase was 0.3 ml/min. These separation conditions were found as optimal with respect to (i) short analysis time, (ii) high separation efficiency, (iii) resolution of QUI and its related impurity in the reference substance.

Table 2. Elution gradient for HPLC-DAD method

Time (min)	**B** (%)	**A** (%)
0.0	10	90
0.5	10	90
1.5	35	65
10.0	35	65
10.5	10	90
15.0	10	90

HPLC-FD method. The conditions optimal for the HPLC-DAD method have not been found to be suitable for HPLC-FD method because of a fluorescence quenching. Optimum separation conditions with respect to the maximum fluorescence response, short analysis time, high separation efficiency and separability were obtained under isocratic reversed-phase conditions using a mobile phase consisting of methanol : acetonitrile: 0.1 mol/l CH_3COONH_4, 45:15:40 (v/v/v) at a flow rate of 0.4 ml/min.

Performance parameters of methods

The CITP-CZE-DAD, HPLC-DAD and HPLC-FD methods with the optimized separation parameters were validated. All resulting statistical data and performance parameters of the methods are given in table 3.

The increased dimensionality of the CITP-CZE-DAD separations in comparison with a single column CE provided favourable conditions in terms of (i) separation efficiency (N) and height equivalent to one theoretical plate (H), and (ii) sample load ability and, consecutively, sensitivity. The obtained values of limit of detection (LOD) and limit of quantification (LOQ) clearly favour the use of the column-coupled CITP-CZE method in ultra trace determination of QUI even if used as a conventional absorbance photometric detection. The LOD and LOQ values obtained from CITP-CZE-DAD method were comparable with the chromatographic methods even though (i) a long-path detection cell (6 cm), or (ii) more sensitive detection (fluorescence) were used in HPLC.

Good linearity of the calibration lines is indicated by the values of coefficient of determination (r^2) that are acceptable and comparable in all the methods tested. Evaluated repeatability is also acceptable and comparable; what is clearly visible from the values of standard deviations (SD) and relative standard deviations (RSD) of, (i) migration time t_m of QUI (RSD_{tm}), (ii) intercept a (RSD_a), (iii) slope b (RSD_b) of the calibration lines of QUI, and (iv) series of the recovery measurements (RSD_{Rec}) of all the methods tested.

The recovery values, calculated for the QUI detection response in the standard (solvent or mobile phase plus additives) and tested (beverage) matrices, were about 100%. This indicated negligible effect of the matrix on the analyte signal, and acceptable accuracy of all the methods tested. The results given by the electrophoretic and chromatographic methods were in a good mutual agreement.

The deliberate altering of the operational parameters in the robustness test resulted in the fluctuations of t_m that were less than ca. 2% (CITP-CZE-DAD) or ca. 3% (HPLC-DAD, HPLC-FD) of the value obtained under the standard conditions. Therefore, it can be supposed that little changes in operational parameters should not influence t_m

significantly and the methods are robust enough. From this point of view, the CITP-CZE-DAD method is presented as a good alternative to the chromatographic methods for its routine use.

Table 3. Performance parameters of CITP-CZE-DAD, HPLC-DAD and HPLC-FD method and parameters of calibration lines of QUI measured in model samples[a]

Parameter	Method		
	CITP-CZE-DAD	**HPLC-DAD**	**HPLC-FD**
t_m [min]	12.350	6.498	10.4520
SD_{tm} [min.]	0.036	0.0681	0.0453
RSD_{tm} [%], n=6	0.290	1.050	0.4300
a [mAU, LU][b]	0.0201	0.2455	5.4396
SD_a [mAU, LU][b]	0.0004	0.0062	0.1697
RSD_a [%], n=6	1.990	2.530	3.1200
b [$mAU.ng^{-1}.ml$, $LU.ng^{-1}.ml$][b]	0.0439	0.8038	0.2418
SD_b [$mAU.ng^{-1}.ml$, $LU.ng^{-1}.ml$][b]	0.0007	0.0063	0.0044
RSD_b [%], n=6	1.590	0.780	1.8400
r^2	0.999	0.9998	0.9994
LOD [$ng.ml^{-1}$][c]	2.290	1.0400	2.1000
LOQ [$ng.ml^{-1}$][c]	7.630	3.4700	7.0000
N	149 000	14 747	13 478
H [μm]	0.940	3.3900	11.1300
Recovery [%][d]	99.4-99.9	98.9-99.6	99.3-100.2
RSD_{Rec} [%], n=6	0.98-1.37	0.85-1.79	1.36-2.11
Robustness (t_m) [%][e]	< 2.04	< 2.71	< 3.12

[a]For the separation conditions see section 3.1., for other working conditions see experimental section. The samples (Kinley): 1000 times diluted beverage for CITP-CZE-DAD; 50 times diluted beverage for HPLC-DAD and HPLC-FD.

[b][mAU] unit corresponds to CITP-CZE-DAD and HPLC-DAD method, [LU] unit corresponds to HPLC-FD method

[c]LOD was calculated as signal-to-noise ratio S/N=3, LOQ was calculated as S/N=10.

[d]Recovery was calculated by spiking of reference matrix (0.5 mmol/l H_2SO_4 and 1 mmol/l NaH_2PO_4 in demineralised water for CITP-CZE-DAD; demineralised water for HPLC-FD and mobile phase – 10% MeOH, 5 mmol/l ammonium formate for HPLC-DAD) and beverage matrix (Kinley) and comparing resulting peak areas of QUI obtained in these different matrices.

[e]Robustness test examined the effect that deliberate variations in operational parameters had on the analysis results (migration time). For the CITP-CZE-DAD method these are concentration of leading (9.5-10.5 mmol/l) and carrier (24-26 mmol/l) cation, pH (±0.1). For the HPLC-DAD method these are pH of the mobile phase (±0.1) and concentration of ammonium formate (4.9-5.1 mmol/l). For the HPLC-FD method these are concentration of ammonium acetate (0.095-0.105 mol/l) and pH (±0.1).

Application in food analysis

The proposed and proved CITP-CZE-DAD, HPLC-DAD and HPLC-FD methods were applied in a quality food control where QUI was determined in selected commercially available bitter drinks (Kinley, Schweppes). Representative records, shown in Figure 1, illustrate the sample profiles characteristic for Kinley. These profiles indicate very good separation selectivity of the separation methods where the beverage matrix impurities (IMP) were separated from the QUI peak. In this way, the determination of the QUI concentration (Table 4.) is more reliable using the separation methods (determination of pure QUI) than the fluorimetric method serving as a reference one (determination of the sum of fluorescing compounds).

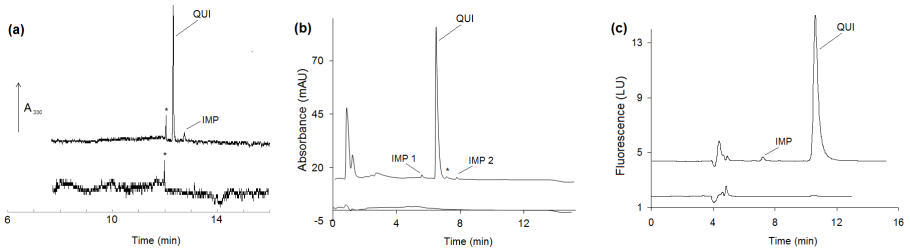

Figure 1. The illustrative records from the QUI separations in the beverage samples (Kinley) by the electrophoretic and chromatographic methods.

(a) Electrophoreogram from the CZE step of the CITP-CZE-DAD combination.
(b) Chromatogram from HPLC-DAD method.
(c) Chromatogram from HPLC-FD method. QUI – quinine, asterisk – impurity from the carrier electrolyte or mobile phase, and QUI standard, IMP – impurity from the selected bitter drink matrix. The beverage samples were diluted 1000 times for CITP-CZE-DAD method and 50 times for HPLC-DAD and HPLC-FD method. For separating and other working conditions see section 2.

Results from the determination of QUI in the bitter drinks, based on the peak area calculations, are given in Table 4. These results confirmed high precision and accuracy of the CITP-CZE-DAD method in comparison with the chromatographic methods. The differences in the QUI concentrations in the beverages obtained by the CITP-CZE-DAD method and HPLC-DAD, HPLC-FD methods, as given in Table 4, ranged in the interval of 1.54-4.90%. These differences clearly indicated a good accuracy of the two-dimensional electrophoretic technique. The results given in Table 4 confirmed the fact that the concentration levels of QUI are acceptable with respect to the manufacturer declaration and/or the health authority's recommendation.

Table 4. Determination of QUI content in bitter drinks by the CITP-CZE-DAD, HPLC-DAD, HPLC-FD methods and Fluorimetry [a]

Method/Parameter	Kinley	Schweppes
CITP-CZE-DAD method		
Found QUI concentration [mg/l]	38.45	56.17
RSD [%], n=6	0.93	1.14
PCC[b]	0.9995	0.9986
HPLC-DAD method		
Found QUI concentration [mg/l]	40.43	57.92
RSD [%], n=6	0.24	0.30
PCC[b]	0.99704	0.99015
HPLC-FLD		
Found QUI concentration [mg/l]	39.92	57.05
RSD [%], n=6	1.24	1.12
Fluorimetry		
Found QUI concentration [mg/l]	43.21	59.96
RSD [%], n=6	1.73	1.49

[a]For the separation and other working conditions as well as for the processing procedure of the spectra see section 2. The samples were 1000-times diluted (CITP-CZE-DAD method) respectively 50-times diluted (HPLC-DAD and HPLC-FD methods) or 5-times diluted (Fluorimetry) and directly injected into the analyzer.

[b]Homogeneity of QUI spectra was expressed via Pearson's correlation coefficients (PCCs) (Miller, 1993). The value of PCC higher than 0.99 is assumed to provide an acceptable certainty in a confirmation of the identity of the analyte (Strašík et al., 2003), i.e. a match of the tested (QUI in beverage) and reference (QUI in demineralised water) spectrum.

A reliability of the determination of QUI in the beverages was supported (i) by the spectral evaluation of the QUI peak (CITP-CZE-DAD, HPLC-DAD) or (ii) selective fluorescence response (HPLC-FD). DAD spectra, shown in Figure 2a,b, revealed the satisfactory purity of the QUI zone/peak and, by that, confirmed an appropriate separation selectivity of the separation methods for the beverage samples. Homogeneity of the QUI spectra obtained from the real beverage samples was expressed via PCCs, for the results see table 4. Here, the matching factors were higher than 0.99. The spectral characterization was applied also for unknown zones (IMP) present in the separation profiles of the beverages (see Figure 1a,b for Kinley). The reference QUI spectra (2a,b) were compared with IMP spectra (Figure 2c,d) and the results were expressed via corresponding PCC values, ranging in the interval of 0.8954-0.9060. These relatively high PCC values along with the excellent separation selectivity of the CITP-CZE and HPLC methods can still indicate, with a good probability, a structural relationship of IMP and QUI. From this one can assume that IMP can be, for example, a degradation product of QUI created during the beverage storage. This application highlighted suitability of the CITP-CZE-DAD method for its routine use in quality food control, similarly to the HPLC-DAD and HPLC-FD methods.

Moreover, the benefits of two-dimensional capillary electrophoresis, as described particularly in the previous sections, are apparent.

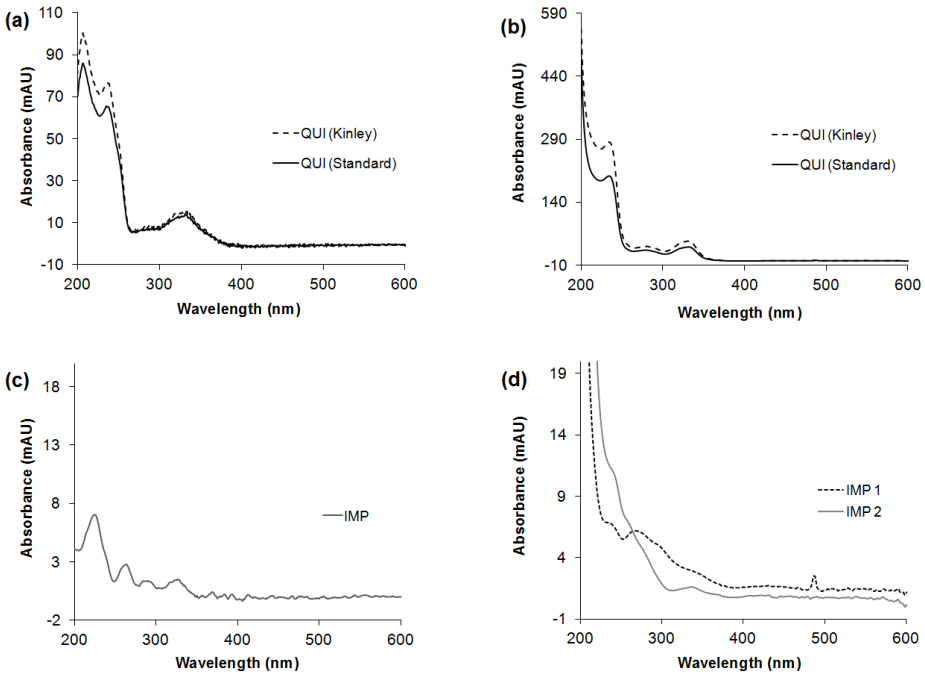

Figure 2. Absorption spectra from DAD detectors hyphenated with electrophoretic and chromatographic techniques.

Spectra of QUI:

a) QUI reference substance and QUI contained in the bitter drink Kinley obtained from the CITP-CZE-DAD method.
b) QUI reference substance and QUI contained in Kinley obtained from the HPLC-DAD method.

Spectra of impurities:

c) Impurity contained in Kinley obtained from the CITP-CZE-DAD method.
d) The impurities contained in Kinley obtained from HPLC-DAD method. For separating and other working conditions see Figure1.

CONCLUSION

The present work illustrated a solid position of the CITP-CZE-DAD method in the group of high performance separation methods. The results obtained from CITP-CZE-DAD, HPLC-DAD and HPLC-FD methods for the determination of QUI in beverages were consistent and highly reliable, supported by specific (spectral, fluorescence) detection responses. The advantages of the CITP sample pretreatment on-

line coupled with the CZE separation and spectrophotometric DAD detection for the highly reliable and effective analysis of QUI in real multicomponent ionic matrices were demonstrated. The on-line CITP-CZE combination enabled (i) the performance of the ultratrace analyses with (ii) increased separation selectivity/separability, and (iii) simplified sample preparation and analytical procedure.

In addition, an integral part of this hyphenated method is the simultaneous control of reliability of the results through the spectral evaluation of the separated electrophoretic zones using the on-capillary spectrophotometric DAD detector. The spectra recorded during the CITP-CZE separation process reflected (i) a purity of marked (known) electrophoretic zones, and (ii) basic structural match between a compound in an unknown zone and the reference standard. Chemometric methods including processing (background correction and smoothing) and comparing (via PCC) of the spectra represented a powerful tool for an objective evaluation of the recorded spectra that can be of a high importance especially when analysing ultra-trace analyte concentrations.

Excellent performance parameters of the CITP-CZE-DAD method and its successful application in the food control area clearly illustrated its potentialities in the reference as well as routine laboratories. Moreover, this hyphenated method is suitable for the automation and miniaturization, and it has promising potentialities also for more complex matrices. Thus, the CITP-CZE-DAD approach is presented as a pragmatic solution (rapid, simple, cheap, available, and highly reliable) to the well-established analytical techniques used in advanced food analysis.

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Porovnanie elektroforézy so spojenými kolónami s metódami kvapalinovej chromatografie v analýze potravín s obsahom chinínu

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Práca prezentuje porovnanie elektroforézy so spojenými kolónami s metódami kvapalinovej chromatografie v analýze potravín s obsahom chinínu (QUI). Kapilárna zónová elektroforéza (CZE) spojená on-line s kapilárnou izotachoforézou (CITP) a spektrofotometrickým detektorom s diódovým poľom (DAD) na báze optických vláken bola porovnávaná s (i) vysokoúčinnou kvapalinovou chromatografiou (HPLC) s DAD detekciou a (ii) HPLC metódou s fluorescenčnou detekciou (FD). V uvedených metódach boli porovnávané validačné parametre a stanovená koncentrácia QUI v nápojoch. Koncentrácia QUI vo dvoch vybraných horkých nápojoch stanovená CITP-CZE-DAD metódou bola v dobrej zhode s koncentráciami nameranými HPLC-DAD a HPLC-FD metódami. CITP-CZE-DAD metóda navyše dokázala, podobne ako chromatografické metódy, oddeliť potenciálne QUI príbuzné nečistoty od píku QUI. CITP-CZE-DAD metóda poskytla vynikajúce validačné parametre porovnateľné (presnosť, správnosť, LOD, robustnosť) alebo dokonca lepšie (separačná účinnosť) ako tie získané z chromatografických metód. Navyše efektivita elektroforetickej metódy bola vyššia berúc do úvahy cenu analýzy (zariadenie, spotreba separačných systémov), environmentálne aspekty (organické *vs*. vodné rozpúšťadlá), on-line predúpravu vzorky (CITP prekoncentrácia a vyčistenie vzorky vhodné i pre komplexnejšie matrice). Vzhľadom na získané výsledky bola CITP-CZE-DAD metóda osvedčená ako rutinná automatizovaná metóda pre vysoko spoľahlivú kontrolu kvality potravín.

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