Separation and Determination of charged drugs in aqueous and biological media using capillary zone electrophoresis coupled to different detectors

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□ ABSTRACT □

In order to determine the pharmacokinetics and pharmacodynamics profiles of drugs after administration by varying routes, capillary electrophoresis coupled to different detectors, as a simple alternative analytical method, was developed to separate and determine both the anticholinergic (Glycopyrronium Bromide; the cationic substance) and the modified gamma cyclodextrin (Sugammadex; the anionic substance) in water and in human biological media; urine and plasma. An excellent linear relationship between the peak areas and the corresponding concentrations was achieved, and that is confirmed by the value of the correlation coefficient ($R^2 > 0.99$). The detection for glycopyrronium bromide was in micrograms per millilitre sensitivity limit (using ultraviolet detector) after a short time of injection. The samples were easily pre-treated; urine samples were diluted with water and plasma ones were treated with acetonitrile. The method showed good reproducibility with RSD < 0.28% for migration time and < 0.92% for peak area at different media. Since sugammadex is a non-active ultraviolet substance, there is no possibility to be directly detected using an analytical instrument coupled to ultraviolet detector. In this study, it was determined easily and rapidly using contactless conductivity detector in water and human urine samples.

Keywords: Capillary electrophoresis, Sugammadex, Glycopyrronium bromide, contactless conductivity detector

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فصل وتحديد الأدوية المتشردة في الأوساط المائية والبيولوجية باستخدام الرحلان الكهربي الشعري مقترن بكواشف مختلفة

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□ ملخّص □

من أجل تحديد ملامح الحرائك الدوائية والديناميكية الدوائية للأدوية بعد تناولها بطرق مختلفة، تم تطوير الرحلان الكهربائي الشعري المقترن بكواشف مختلفة، كطريقة تحليلية بديلة بسيطة، لفصل وتحديد كل من مضادات الكولين (بروميد الجليكوبيرونيوم؛ المادة الكاتيونية) و الجاما سيكلوديكسترين المعدلة (سوغاماديكس؛ المادة الأنيونية) في الماء وفي الوسائط البيولوجية البشرية؛ البول والبلازما. أظهرت جميع النتائج أن هناك علاقة خطية ممتازة بين المساحة تحت سطح منحني والتراكيز المقابلة لها، وهذا ما تؤكده قيمة معامل الارتباط (0.99). تم الكشف عن بروميد الجليكوبيرونيوم بالميكروجرام لكل ملليلتر (باستخدام كاشف الأشعة فوق البنفسجية) بعد فترة قصيرة من الحقن.

تمت معالجة العينات مسبقًا بسهولة؛ تم تخفيف عينات البول بالماء وتم علاج عينات البلازما باستخدام الأسيتونيتريل. نظرًا لأن سوجاماديكس مادة غير ممتصة للأشعة فوق البنفسجية، فلا توجد إمكانية لاكتشافها مباشرة باستخدام أداة تحليلية مقترنة بكاشف للأشعة فوق البنفسجية. وفي هذه الدراسة، تم تحديدها بسهولة وسرعة باستخدام كاشف الموصلية بغير تلامس في عينات الماء والبول البشري.

الكلمات المفتاحية: الرحلان الكهربائي الشعري، سوغاماديكس ، بروميد الجليكوبيرونيوم، كاشف الموصلية بغير تلامس

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Introduction

Glycopyrronium bromide (GLB) is a synthetic quaternary ammonium compound (Figure 1). According to its anti-cholinergic action, it has different pharmacological effects. It is used preoperatively to reduce gastric and salivary secretion and during anaesthesia, it is given intravenously for reversing neuromuscular blockage [1]. some studies show the effectiveness of topical GLB (roll-on, creams and solutions) [2] and oral dosage form [3] in the treatment of gustatory hyperhidrosis. In order to investigate the pharmacokinetic and pharmacodynamics profiles of drugs after administration by varying routes, studies should point their efforts to separate and determine GLB in different media either aqueous one or biological one. Previously, in human plasma, GLB was determined by capillary column gas chromatography with nitrogen-sensitive detector. The method was based on ion-pair extraction of the drug [4]. Liquid chromatography-mass spectrometry methods were validated and developed for detection GLB and different quaternary ammonium drugs (QAD) in human and equine biological fluids [5-8]. The sample preparation involved QADs to be extracted from biological fluids using solid phase extraction technique procedures. Capillary electrophoresis methods were also performed to separate and determine different quaternary ammonium compounds [9-11]. Eight quaternary ammonium drugs were analyzed by capillary electrophoresis – mass spectrometry after extraction from horse urine by ion pair extraction method [12].

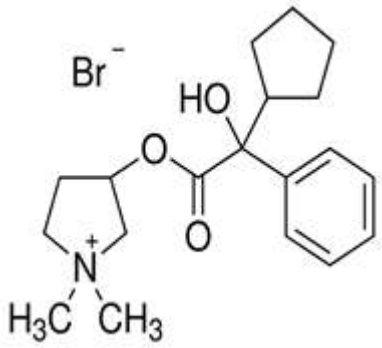

Figure 1; chemical structure of glycopyrronium bromide

In postsurgical treatment, sugammadex (SUG) that is a modified γ-cyclodextrin (CD) (Figure 2) forms an inclusion complex with rocuronium bromide, causing the neuromuscular blocking agent to have an opposite effect [13]. Sugammadex was determined before in human plasma, urine and dialysate using high-performance liquid chromatography/tandem mass spectrometry after being extracted using an expensive technique (SPE) [14].

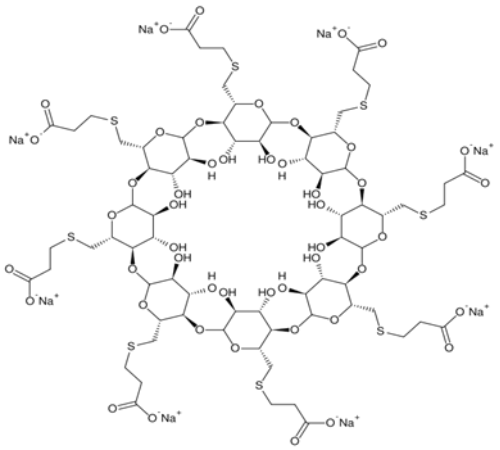

Figure 2; chemical structure of sugammadex

In general, procedures were developed for analysis different drugs in biological samples (plasma, urine, bile, saliva, etc.) using capillary zone electrophoresis (CZE) [15-19].
The objective of this study was to develop an alternative, robust and non-complicated analytical method to determine the cationic drug (GLB) and the anionic drug (SUG). The success was achieved by choosing the CZE, the simply operative technique. At the level of micrograms in millilitre, GLB was detected within a short time of 4 minutes. On the other hand, and as an advantage over the previously mentioned methods, separation of GLB of human biological fluids (urine and plasma) was easily carried out by a simple treatment with water and acetonitrile, respectively. Since SUG has a non-detectable UV absorbance, no possibility exists to detect SUG by using an analytical instrument of ultraviolet (UV) detector. Contactless conductivity detector in capillary electrophoresis (CE-C^4D) which has recently a wide application in analysis of organic ions and inorganic ones, were chosen for determination and quantification of SUG.

Experimental part

Materials

Pure substance of GLB from RIEMSER Pharma GmbH (Greifswald, Germany) and SUG, Bridion®100mg/ml, i.v. (intravenous) from N.V. Organon (Oss, Nether-lands) were used. Acetonitrile was from VWR (Fontenay-sous-Bois, France). For background electrolytes, potassium hydrogen-phosphate, potassium di-hydrogen-phosphate, morpholine ethanesulfonic acid (MES) and L-histidine (His) were used.

Instrumental Conditions

A 3D CE system (Waldbronn, Germany) was used to perform CE experiments for GLB, and a diode array detector (190 to 600 nm) was coupled to it. Instrument control, data acquisition, and data analysis were conducted through the use of a CE ChemStation that had an HP Vectra 486/66U workstation. Windows software was utilized to control the system, which was modified to run on the HP system. GLB was detected at 195 nm. The fused silica capillary was 64.5 cm in length and had an internal diameter of 50 millimeters. The distance to the detector was 56 cm. After washing the capillary with 1 N NaOH at 40° C for 15 minutes, it was then rinsed with distilled water for 15 minutes. The capillary was rinsed with 0.1 N NaOH for 3 minutes before being rinsed with distilled water for 2 minutes for each run. For 5 minutes, the buffer solution was used to rinse the capillary during the final step. The temperature was kept at 25° C and the separation voltage was 20 kV. Each sample was injected at 50 mbar for 9 seconds and the analysis run was repeated

three times. CE experiments for SUG were performed using a contactless conductivity detector coupled to CE system. The uncoated fused silica capillary with an internal diameter of 50 µm and 75 µm for water and human urine samples, receptively, was used. The temperature was kept at 27° C and the separation voltage was -20 kV. Pressure of 50 mbar was applied at injection step. And in order to optimize the conditions for shorter migration time, pressure of 80 mbar was applied at the measuring step. For each sample, the analysis run was repeated three times.

Buffer Preparation

The preparation of 10 mM of phosphate buffer for GLB measurements required dissolving of 1.24 g potassium hydrogen-phosphate and 0.39 g potassium di-hydrogen-phosphate in 1 L distilled water with pH 7.4. A 0.45 µm syringe filter was used to filter the buffer solution and to degas it using ultrasound for a minimum of 15 minutes before use.

For SUG measurements, morpholine ethanesulfonic acid (MES) as zwittronic buffer which is characterized by its low conductivity was used. MES was prepared in mixture with L-histidine (His) to adjust the pH of buffer to desired value (5.6). 20 mM and 10 mM of zwittronic buffer were prepared for water and human urine samples, respectively.

Sample Preparation

A set of standard GLB samples was prepared by diluting suitable amount of stock sample with distilled water to get the desired concentrations (1 - 160 µg/ml). For human urine samples spiked with known concentrations of GLB, the samples were diluted with 3 ml distilled water (4 ml total volume) and directly injected after filtration by 0.45 µm syringe filter. In order to precipitate the protein component, each 1 ml of defined amount GLB containing the human plasma sample was diluted to 3 ml total volume by acetonitrile and was centrifuged at 2000 rpm for 20 min. The supernatant was obtained and filtered before use.

A series of SUG sample solution in water was prepared in concentrations between 0.05 mg/ml and 1 mg/ml. For human urine samples spiked with known concentrations of SUG, the samples were diluted with water in ratio of (1:5). Human plasma samples spiked with SUG were treated with acetonitrile. For Another set of human plasma samples spiked with SUG, pre-treatment procedure using solid phase extraction (SPE) with Isolute HAX sorbent material per column (mixed mode of non polar and strong anion exchange) was applied. 1 ml of Methanol/water (50:50) was used for elution step.

Recovery Study

GLB was spiked into three plasma samples at 40 and 160 µg/ml. The samples were analyzed and the peak areas that resulted were compared to those that resulted from aqueous solutions at the same concentrations.

Results and Discussion

GLB results

The freely soluble property of the drug and its UV absorption made the capillary electrophoresis suitable analysis technique for separation and detection of GLB in water and biological fluids. Within 4 minutes from injection in BGE containing standard fused silica capillary, the drug peak was observed. The cationic, permanently ionized drug over a wide range of pH buffer solutions at 25 °C has a positive, effective mobility (2.06×10^{-4} $cm^2.V^{-1}.s^{-1}$). The calibration curve of peak areas in water was linear (Table 1) with a correlation coefficient of $R^2 = 0.999$. A very good reproducibility of the method was

concluded from the RSD values (0.07 %) and (0 %) of migration time and peak area for drug dissolved in water, respectively.

Table 1; results of GLB analysis in water and biological fluids by CE

sample	Linear equation	R^2*	RSD (t) (%)	RSD (p) (%)	Detection limit (µg/ml)
Water	Y= -1.3665+1.2645X	0.9998	0.07	0	0.5
Urine	Y= -0.252+1.2603X	0.9999	0.14	0.92	1
Plasma	Y= 2.757+1.2202X	0.9997	0.28	0.8	1

(t) Relative standard deviation of the migration times (n = 3)
(p) Relative standard deviation of the peak areas (n = 3)
*Correlation coefficient

Very good separation was obtained for GLB in urine after direct injection of diluted samples (Figure 3). The separation procedure was easy, simple and effective in comparison to more complicated methods mentioned previously.

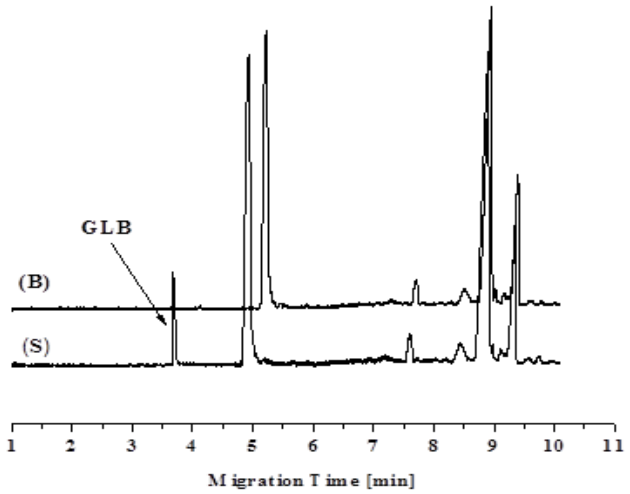

Figure 3; Electropherogram of blank human urine
(B) and 40 µg/ml GLB containing urine sample (S); analysis conditions described previously

GLB was successfully separated from plasma components (Figure 4) in clear sharp peak and quantitatively detected. The samples were treated with acetonitrile (1:3) prior to injection as previously described. Good separation and detection results were obtained after this treatment; linear was the calibration curve of treated plasma samples (Table 1) with a correlation factor of R^2 = 0.999. In comparison to standard GLB samples of same concentrations, the mean recoveries (n = 3) from spiked plasma samples were 99.4% at 160 µg/ml and 103.02% at 40 µg/ml concentration of GLB.

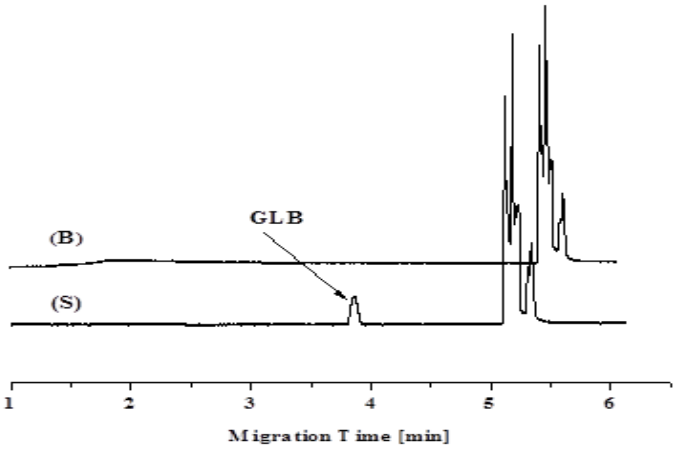

Figure 4; Electropherogram of blank human plasma
(B) and 10 µg/ml GLB containing plasma sample (S); analysis conditions described previously.

The relative standard deviations of peak area and migration time for GLB (10 µg/ml) spiked in biological media different media were calculated in order to control the method reproducibility. Good reproducibility was again confirmed (Table 1). The method showed detection sensitivity in the limit of micrograms per millilitre.

SUG results

The drug peak of freely soluble and non-UV absorbable anionic substance spiked in water samples was successfully detected within approximately 2 minutes after injection in zwittronic buffer containing uncoated fused silica capillary coupled to contactless conductivity detector. A plot of the peak areas versus the corresponding calibration concentrations of SUG showed a linearity with correlation factor of 0.999 (Figure 5). The limit of detection was 0.01 ± 0.0005 mg per ml.

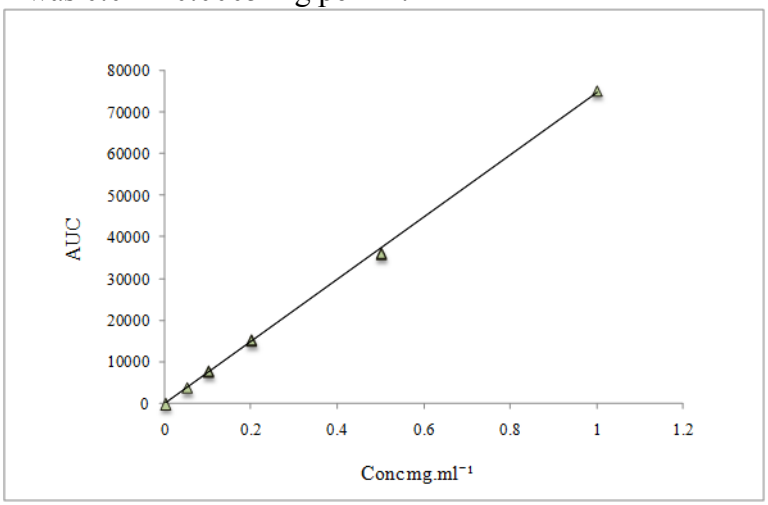

Figure 5; a linear calibration curve of SUG freely dissolved in water samples (n=3)

SUG was successfully separated from human urine components as shown in Figure 6. SUG was quantified in human urine samples that were diluted with water in ratio (1:5). A plot of the peak areas versus the corresponding calibration concentrations of SUG showed a

linearity with correlation factor of 0.998 (Figure 7). The limit of detection was 0.6 ± 0.01 mg per ml. The analysis method showed good reproducibility for peak areas (RSD = 1%) and migration times (RSD = 0.6%) through direct injection of samples without need to be treated or extracted before measurement. That gives this method advantages of being simple, not expensive especially with the scarcity of detection possibilities for the problematic drug (SUG) by other methods. On the other hand, the limit of detection in urine samples was good for drug like SUG which is rapidly cleared and mainly unchanged through renal excretion, but of course not enough in case of comparison to ones obtained by high-performance liquid chromatography/tandem mass spectrometry [14].

For a sample of human plasma, using acetonitrile for protein precipitation was not suitable for SUG since the later was not detected in filtrate part. And that could be attributed to complex formation between CD and one of plasma components. The efforts did not success in detection of SUG through pre-treatment procedure using SPE. That could be attributed to either not enough amount of SUG was eluted to be detectable (sensitivity problems) or the amount was completely stay at column sorbent material.

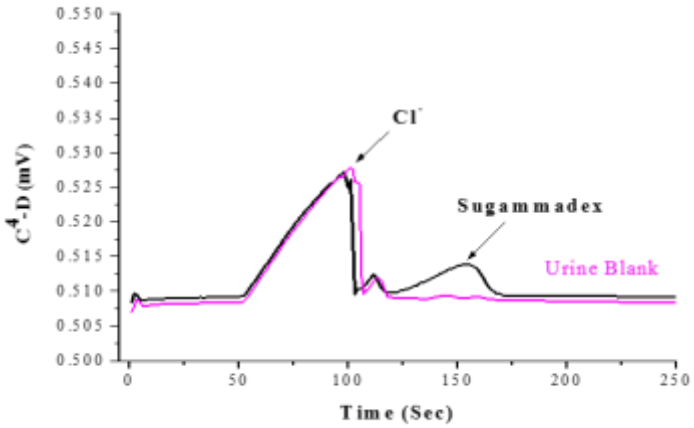

Figure 6; Electropherogram of SUG in human urine sample and blank one

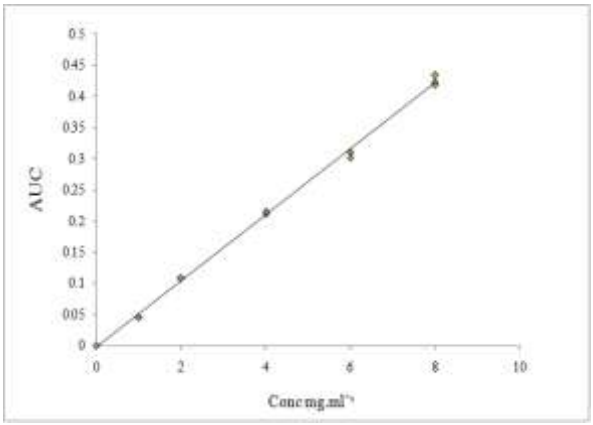

Figure 7; a linear calibration curve of SUG spiked in human urine samples (n=3)

Conclusion

Analyses of GLB and SUG in water and biological fluids were successfully done using CZE with different detectors. An easy-to-handle operation, short analysis time and good reproducibility were reported as method advantages. Furthermore, special or complicated techniques such as solid phase extraction or ion pair extraction, were not needed for pre-treatment the samples in biological fluids. Consequently, clinical studies interested in this technique can find it useful for detecting GLB and SUG in biological fluids.

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