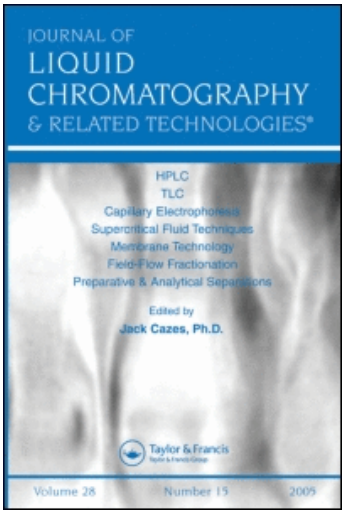

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Determination of Azide and Bromide Ions by Direct Detection Using Capillary Electrophoresis

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Abstract: The purpose of the present work was the development and validation of a simple, rapid, sensitive, and reliable method for the direct determinations of azide and bromide anions in valsartan and baclofen samples, respectively, based on capillary electrophoresis. Azide and bromide ions were determined using nitrate as an internal standard. Azide and nitrate were separated using a fused silica capillary column of total length 64.5 cm × 50 μm, and bromide and nitrate by a 104 cm × 50 μm capillary column. The background electrolyte was an inorganic anion buffer of pH 7.7, and UV detection at 245 nm was used for the determination. The detection limits for azide and bromide ions were 0.5 and 0.3 μg mL^{-1}, respectively, with S/N ratios of less than 3.0 and the quantitation limits were 1.5 and 1.0 μg mL^{-1}, respectively, with S/N ratios of less than 10.9. Excellent linearity was observed in the range from 5 – 30 μg mL^{-1} and 1 – 15 μg mL^{-1}, for azide and bromide ions, respectively. Quantitative imprecision in intra-day (n = 5) and inter-day (n = 5) experiments was always within R.S.D. of <5%. Recovery was quantitative throughout the range of linearity of the method. Mean recovery values were ranging from 97.7 to 99.8% for both anions. The method was applied for the determination of azide and bromide contents in the batch samples of valsartan and baclofen, respectively. The developed method was validated in accordance with the ICH guidelines and proved to be suitable for its intended use.

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Keywords: Azide and bromide, Capillary electrophoresis, Determination of anions, Valsartan and baclofen samples

INTRODUCTION

Organic acids and anions play an essential role in both environmental and biological processes. Determination of these molecules is important for food and beverages, pharmaceutical and environmental industries, as well as for control and research laboratories. The analysis of small organic and inorganic anions is of interest in a wide range of industries, and pharmaceutical, clinical, and forensic sciences.[1,2]

Various methods have been developed for the determination of inorganic anions including atomic absorption spectrometry,[3] flow injection spectrophotometry,[4] catalytic kinetic spectrophotometry,[5] potentiometry,[6] inductively coupled plasma mass spectrometry,[7] ion chromatography (IC),[8] and capillary electrophoresis (CE).[9–11] The advantage of the chromatographic techniques such as CE and IC is the ability to quickly profile and quantitative anionic species. CE has recently become a powerful analytical technique that can provide high resolution efficiency and a complementary technique to chromatographic methods. Separation speed, superior separation efficiency, good sensitivity, accuracy, precision, excellent robustness,[12] and direct injection of samples without labor intensive sample preparation are the major advantages of the CE method for the determination of real samples. In addition, in the case of samples showing disparate levels of analytes, CE was found to be more robust than IC.[13] Simultaneous determination of inorganic anions and carboxylic acids has often been achieved in CE with direct UV detection.[14]

Azide salts are highly toxic and are important for developing methods for the determination of azide ions in different samples. Azide was analyzed in protein samples using anion exchange chromatography with electrochemical detection;[15] A CE method[16] with a photodiode array detector has been used to determine azide as the 3,5-dinitrobenzoyl derivative in drink samples and another CE method with indirect spectrophotometric detection was applied to detect azide in forensic specimens from two suicide victims by Hortin et al.[17] There are several methods reported for determination of bromide, including catalytic kinetic spectrophotometric determination in drug samples,[18] chemiluminescence detection in seawater,[19] photometric determination in oil,[20] and liquid chromatographic determination in cereals, fruit, vegetables, and blood.[21] CE has also been used for determination of bromide in a local anaesthetic hydrochloride with direct UV detection,[22] raw and drinking waters,[23] and in human serum.[24]

Valsartan is an angiotensin II receptor antagonist, commonly called "ARB" which stands for angiotensin receptor blocker, acting on the AT_1

subtype. In the US, valsartan is indicated for treatment of high blood pressure, of congestive heart failure, and post myocardial infarction. Wang et al.[25] reported some efficacy in the use of valsartan in the treatment and prevention of Alzheimer's disease. In the synthesis of valsartan, the intermediates containing a cyano group are converted to the corresponding intermediates containing a 5-tetrazolyl group, thereby reducing the possibility of contaminating the resulting valsartan with tin or azide derivatives. Synthetic valsartan is in fact substantially pure, and in particular substantially free from tin or azide derivatives. The expression "substantially pure" means having a purity degree equal to or higher than 99%. The expression "substantially free from tin or azide derivatives" means having contents in said contaminants equal to or lower than 20 ppm.

Baclofen, most common brand names of Kemstro and Lioresal, is a derivative of γ-amino butyric acid (GABA) primarily used to treat spasticity. Baclofen is used for the treatment of spastic movements, especially in the instance of spinal cord injury,[26] spastic diplegia, multiple sclerosis, amyotrophic lateral sclerosis and trigeminal, and gloss pharyngeal neuralgias. The literature survey indicates that the biological activities of γ-phenyl-GABA include anticonvulsant,[27] antiepileptic,[28] antistress,[29] and antihypoxic,[30] antihypertensive,[31] and analgesic activities.[32] During the synthesis of baclofan samples, one of the reagents contains bromide.[33,34] Consequently bromide content should be equal to or lower than 20 ppm in baclofan samples.

The mentioned methods for the determination of azide and bromide ions in different samples like proteins, drinks, sea water, vegetables, etc., necessitate the use of some reagents for determination. The method developed in this work does not require any reagents for determination of azide and bromide ions. However, from the above discussion on valsartan and baclofen, it is significant to develop sensitive methods for the determination of azide and bromide ions in these samples, respectively. The aim of the present study was to develop a simple, rapid, sensitive, and reliable CE method for direct determination of azide ion in valsartan samples and bromide in baclofen samples using nitrate ion as an internal standard. The method was validated in terms of specificity, detection limit, quantitation limit, linearity, accuracy, precision, and repeatability, in accordance with the ICH guidelines.[35,36]

EXPERIMENTAL

Chemicals

Inorganic anion buffer (pH 7.7) was purchased from Agilent Technologies (Waldbronn, Germany). Sodium salt of azide, potassium salts

of bromide, and nitrate were purchased from Sigma-Aldrich. Valsartan and baclofen samples were obtained from Matrix labs Pvt. Ltd (Hyderabad, India). The standard solutions of azide and bromide were prepared from their corresponding salts. Samples were dissolved in deionized water and sonicated for 10 min, and filtered with 0.45 μm membrane filters. Deionized water was obtained with an Elgacan filtration system.

Apparatus

Separation was performed with an Agilent G1600AX CE injection system (Waldbronn, Germany) in combination with a variable wavelength UV-visible detector. The wavelength was set at 245 nm. Sample injection was carried out by pressure (50 mbar, 150 sec) at the cathodic side of the capillary. The analysis voltage was −30 kV. The fused silica capillaries for the separation experiment were 64.5 cm × 50 μm and 104 cm × 50 μm, respectively, for azide and bromide ions. The distance to the detection window was 50 cm. The measurement was performed at 20°C. The analysis time was 12 min. Samples were dissolved in internal standard and diluted, and filtered using 0.45 μm nylon filtered paper. Automated capillary rinsing, sample introduction, and execution of the electrophoresis runs were controlled by a personal computer. Data processing was carried out with the Agilent chemstation software.

RESULTS AND DISCUSSION

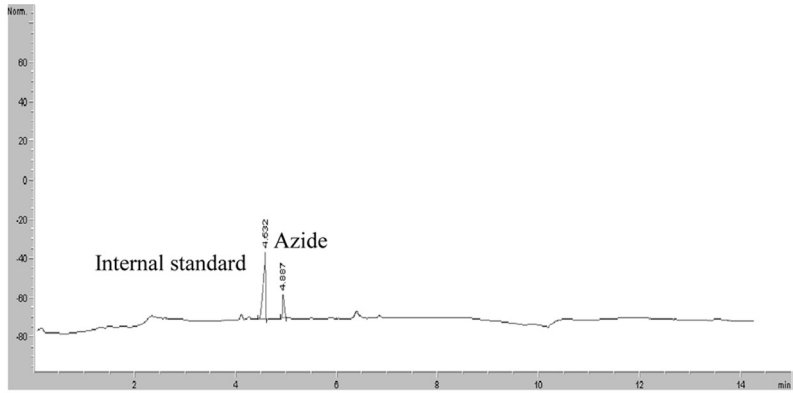
Specificity

The capillary electrophoresis method for the determination of anion concentrations is based on differential migration and separation of anions in an electric field due to their different electrophoretic mobility. In the present method, identification and quantitation of the anions were performed by direct detection by measuring the UV absorption at required wavelength. As an initial part of the method, azide and bromide ions are separated by using an inorganic anion buffer of pH 7.7 as the background electrolyte solution. The solutions of azide and bromide were prepared freshly and analyzed individually at a concentration of 10 and 90 μg mL^{-1}, respectively, with nitrate as an internal standard. Table 1 presents the migration times of azide and bromide ions and are shown to be 13.24 and 4.88 min., respectively. The electropherogram was shown in Figure 1, for the separation of azide and nitrate (Figure 1a) and bromide and nitrate (Figure 1b), respectively. The migration time reproducibilities of these anions are calculated in terms of standard deviation for

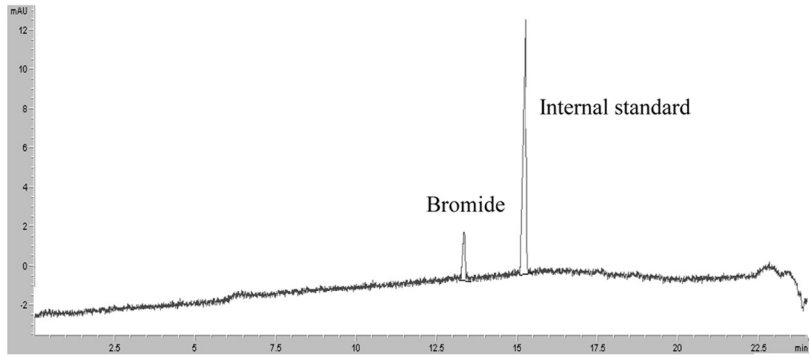
Table 1. Migration times of azide and bromide ions

Compound	Migration time (min)	Standard deviation (n = 6)
Azide	4.88	0.15
Bromide	13.24	0.21

six injections as 0.15 and 0.21, respectively, for azide and bromide ions (Table 1). Also, the migration retention times measured for spiked anions were comparable to the standard anions data; the differences were smaller than 0.2 min. This indicates the method is specific for the determination of azide and bromide ions, individually.



(a)



(b)

Figure 1. Typical electropherogram of (a) azide, and (b) bromide with nitrate internal standard.

Table 2. Quantitative performance

Compound	Calibration range (μg mL^{-1})	Calibration curve	r^2	LOD (μg mL^{-1})	S/N Ratio	LOQ (μg mL^{-1})	S/N Ratio
Azide	5–30	$Y = 5.2186x + 0.1174$	0.9992	0.5	2.9	1.5	10.9
Bromide	1–15	$Y = 9.1334x + 0.3005$	0.9994	0.3	3.0	1.0	9.5

Limit of Detection

The limit of detection (LOD) is determined by calculating the signal to noise ratio and by comparing test results from samples with known concentrations of analyte with those of blank samples, and establishing the minimum level at which the analyte can be reliably detected. The LOD is calculated from the signal to noise (S/N) ratio. The results obtained are listed in Table 2, and the representative electropherogram of azide ion at the LOD level was shown in Figure 2. The LOD's for azide and bromide ions were found to be 0.5 and 0.3 μg mL^{-1} with S/N ratios of 2.9 and 3.0, respectively.

Limit of Quantitation

The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The LOQ is calculated from the signal to noise ratio. The LOQ values for azide and bromide ions were found to be 1.5 and 1.0 μg mL^{-1} with S/N ratios of 10.9 and 9.5, respectively (Table 2). A representative LOQ electropherogram for the azide ion was shown in Figure 3.

Linearity

Calibrations were done for different spiked concentrations of azide and bromide ions separately and are analyzed. Linear responses were

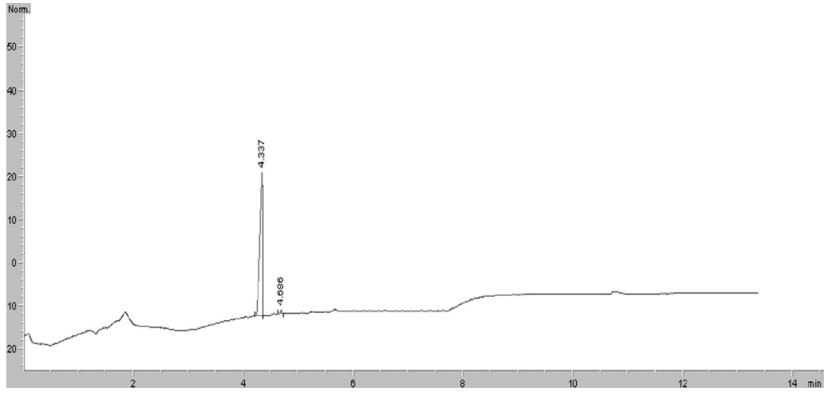


Figure 2. Electropherogram of azide ion at LOD level.

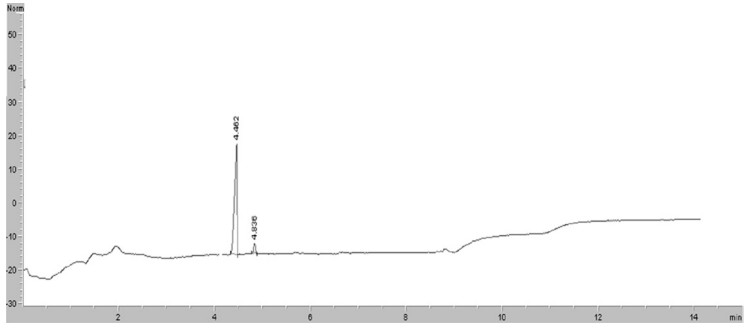


Figure 3. Electropherogram of azide ion at LOQ level.

obtained between the concentration range, 5–30 μg mL^{-1} and 1–15 μg mL^{-1}, for azide and bromide ions, respectively, with a regression coefficient (r^2) higher than 0.999. Each calibration equation was fitted by the linear regression equation $y = mx + c$, where y was the signal peak area ratio between the analyte and IS and x is the concentration of the spiked analyte ion. Figure 4 shows the calibration curves of azide and bromide ions, and slopes, intercepts, and regression coefficients were summarized in Table 2

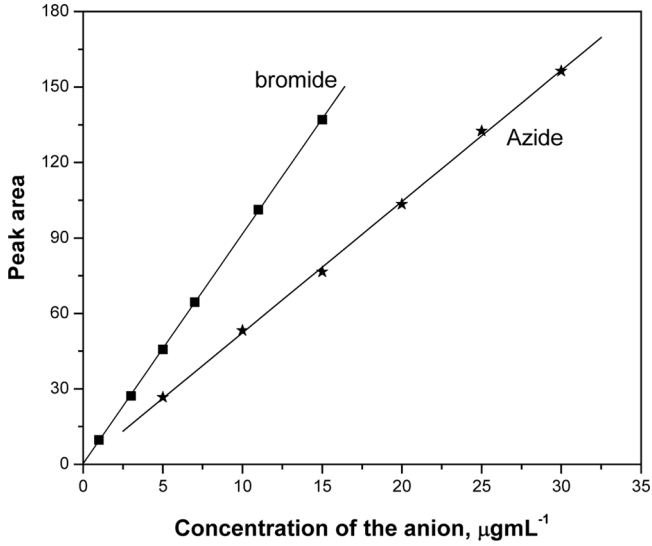


Figure 4. Calibration graphs of azide and bromide ions.

Table 3. Intra-day and inter-day accuracy, precision and recovery for quantitation of azide and bromide

				Intra-day repeatability (n = 5)			Inter-day repeatability (n = 5)		
Anion	Spiked conc. ($\mu g\,mL^{-1}$)	Recovery (% Mean ± SD)	RSD (%) (n = 6)	Conc. found ($\mu g\ mL^{-1}$)	RSD (%)	Accuracy (%)	Conc. found ($\mu g\ mL^{-1}$)	RSD (%)	Accuracy (%)
Azide	10	98.7 ± 1.5	1.48	9.8	1.43	98	9.7	4.5	97
	20	99.0 ± 1.1	1.15	9.6	1.26	96	9.8	5.3	98
	30	99.7 ± 0.9	0.91	9.9	1.03	99	10.1	2.1	101
Bromide	5	97.7 ± 1.4	2.15	5.1	1.45	102	4.9	4.2	98
	10	99.8 ± 1.6	1.80	9.8	1.22	98	9.9	5.0	99
	15	98.4 ± 1.6	1.95	15.1	1.05	101	14.9	2.6	99

Precision and Accuracy

In the present work, precision and accuracy of azide and bromide ions were determined by analyzing a solution containing valsartan spiked with an azide ion and baclofen spiked with bromide at approximately LOQ level of the working strength of the active pharmaceutical ingredient (API). The precision and accuracy of intra-day (five successive injections) and inter-day (five successive days) for three different spiked concentrations of low, intermediate, and high spiked levels of both anions were determined and the results are presented in Table 3. The RSD values of both ions for intra-day experiments ranged from 1.03 to 1.45% and for inter-day experiments from 2.1 to 5.5%. The accuracies of intra- and inter-day experiments were ranged from 96 to 102% and 97 to 101%, respectively.

Recovery

The recovery study was carried out for both azide and bromide anions by preparing three different spiked concentration levels, as mentioned in precision and accuracy. The recovery ranges were 98.7 to 99.7% and 97.7 to 99.8% for azide and bromide anions, respectively. Results of recovery tests are shown in Table 3. The average percentage recoveries obtained for azide and bromide ions were 99.13% and 98.64%, respectively.

Batch Analysis

The proposed method was applied for the determinations of azide in valsartan samples and bromide in baclofen samples (five batch samples for each anion) provided by Matrix laboratories. The results of batch analysis show that in all batches, the contents of azide and bromide anions in the corresponding samples were less than their respective detection limits.

CONCLUSIONS

The developed method is accurate, specific, and precise for the individual determination of azide and bromide anions in valsartan samples and baclofen samples, respectively, by capillary electrophoresis with direction detection at 245 nm. This method has marked advantages in terms of feasibility and rapidity in comparison with other analytical methods. The reliability of the method developed was established by good calibration

linearity, excellent relative migration time repeatability, and satisfactory accuracy and recovery for intra-day and inter-day analysis with respect to ICH guidelines. In summary, CE was shown to provide a quick, powerful, economic, and reliable method for the determination of both inorganic anions, and the developed method was useful for fast routine analysis of pharmaceutical samples. The developed method was validated according to the ICH guidelines and proved to be suitable for the intended use.

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