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Original Article

Determination of Aciclovir and its Related Substance Guanine in Bulk Drug and Tablet Preparation by Capillary Electrophoresis

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Abstract

Two Capillary Zone electrophoresis (CZE) methods were developed in this study, one using an acidic buffer (sodium citrate pH = 2.5) and the other using a basic buffer (sodium tetraborate pH = 9.8). The two methods were compared on the basis of repeatability and reproducibility of results and the CZE method developed with the basic buffer was then selected for further studies. The method was fully validated in terms of repeatability [RSD for migration time and peak area of aciclovir at 0.05 mg (nominal concentration) were 0.3-1.0% (x=10), and 1.5-2.6% (n=3), respectively], reproducibility (RSD of peak area = 2.54%, n = 6), linearity over two ranges of aciclovir concentration (0.01-0.07 and 0.05-0.3 mg/ml which resulted in y=2.007x +1.300 and y=0.234x + 0.82, respectively) , limits of detection and quantitation (1×10^{-4} mg/ml and 3×10^{-4} mg/ml, respectively), ruggedness and robustness. The method was applied for the determination of the drug in a commercial tablet preparation (mean recovery value 100.2% w/w) and a commercial injection solution. The method proved to be fast and reliable for quantitative analysis of aciclovir and its related substance in bulk and pharmaceutical dosage forms.

Keywords: Aciclovir; Guanine; Tablet; Assay; Capillary Electrophoresis.

Introduction

Quantitative analytical measurement of drugs is fundamental to the pharmaceutical sciences. The ability of the analytical method to measure drug concentration accurately and precisely is critical, whether the analysis is in biological fluids or in pharmaceutical preparations. Several methods currently are available for determining drug concentration, including spectrophotometry, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme-multiplied immunoassay (EMIT), and chromatographic techniques (TLC, GC, HPLC) (1). The employment of capillary electrophoresis (CE) for the analysis of drugs and pharmaceuticals has been demonstrated in excellent reviews (2-4). The popularity of CE in the pharmaceutical fields has been accelerated by its simplicity, high efficiency and selectivity, and large separation capacity (5-6).

Aciclovir (9-[(2-hydroxyethoxy)methyl] guanine) is an antiviral drug which is used for the treatment and prophylaxis of infections due to herpes simplex and varicella-zoster viruses (7). Guanine, a purine base, is the synthetic precursor, degradation product, and also metabolite of aciclovir (Figure 1). A micellar electrokinetic chromatography(MEKC) method for the quantitation of aciclovir in topical formulations has been reported (8). It is proposed that aciclovir and guanine can be easily separated in both extremes of the pH range using capillary zone electrophoresis (CZE). Thus, two CZE methods were developed in this study, using an acidic buffer (sodium citrate pH=2.5) and a basic buffer (sodium tetraborate pH = 9.8). The two methods were compared on the basis of repeatability of results and the CZE method developed with the basic buffer was them validated and used for determination of aciclovir in tablet dosage form.

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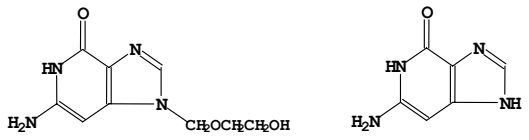

Aciclovir **Guanine**

Figure 1. Chemical structure of aciclovir and guanine

Experimental

CZE conditions/materials

A model 270A Applied Biosystems (ABI) capillary electrophoresis instrument, equipped with Hewlett-Packard model HP3396A integrator was used throughout the study with uncoated fused silica capillaries of 720 mm total length x 0.05 mm I.D. (500 mm to the detector). The temperature of capillary was kept at a relatively constant at 25°C, using a thermostatic oven. In all experiments the detector was set at 0.05 aufs and with a rise time of 1.0 second. Samples were introduced into the capillary by hydrodynamic injection (i.e. applying vacuum at the outlet) and in all experiments, the injection time was 2 seconds (unless otherwise specified). The detection wavelength was 254 nm and temperature was set at 25°C. Other conditions for the two CZE methods developed and used were as follows: a) For the CZE method performed at the acidic pH (2.5), the buffer was sodium citrate 20 mM and the applied voltage was 30 KV) For the CZE method performed at the basic pH, sodium tetraborate was used. Buffer concentration and pH (over the range of 8-10), and the applied voltage were optimized. The optimum conditions were borate buffer 20 mM at pH 9.8 and applied voltage of 25 KV.

Standard and sample solutions

Standard solutions: In all experiments (except in linearity testing) standard solutions of 0.05 mg/ml aciclovir and/or guanine were used. Stock solutions of aciclovir and guanine (1 mg/ml) were prepared separately by dissolving the drug either in HCl (0.1 M) or in NaOH solution (0.05 M) and diluting with the acidic or basic buffer, respectively, to the nominal concentration. The standard solutions used in the linearity assessment were similarly prepared

Sample solutions: Twenty Zovirax tablets (GlaxoSmithKline, UK), each containing 200 mg of the aciclovir were accurately weighed and ground to fine powder and the amount equivalent to one tablet was shaken on the sonic bath for 10 minutes. The mixture was filtered in to a 200 ml volumetric fask and buffer was added to volume (0.05 mg/ml).

Results and Discussion

Mechanism of separation

Aciclovir and its related substance, guanine, are charged at the two extremes of the pH range. Aciclovir (pKa_1=2.27 and pKa_2 = 9.25) (9) and guanine (pKb = 3.2 and pKa = 9.92) (10) are basic substances and are protonated in acidic solutions. They also possess a weak acidic group (enolic proton) in their structure (Figure 1) and therefore, are ionized to negatively charged species at high pH range. Therefore, they can be separated in CZE using either acidic or alkaline buffers. In acidic medium, guanine migrates faster than aciclovir and in alkaline solution, it migrates slower. This is due to the higher charge-to- mass ratio of the guanine. In fact, the two molecules are structurally very similar and the natures of the ionizable groups are very similar, but guanine has a lower molecular weight and thus, has a higher charge-to-mass ratio.

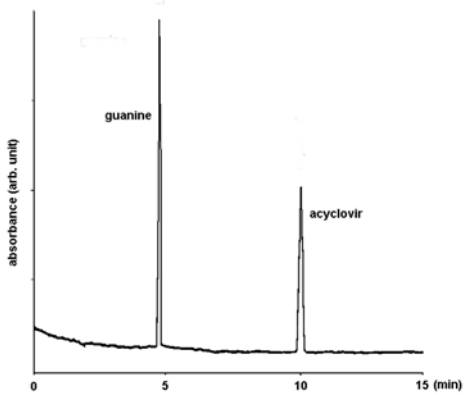

Figure 2. Electropherogram showing separation of aciclovir and guanine in low pH buffer.Conditions: capillary 720 mm (500 nm to detector), buffer sodium citrate 20 mM pH=2.5, voltage 30kV, wavelength 254 nm, temperature(25°C)

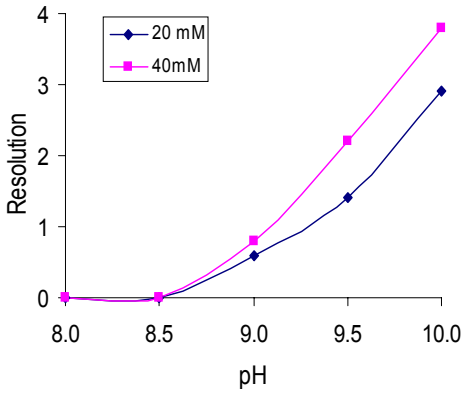

Figure 3. Resolution of aciclovir and guanine at two levels of the buffer concentration against pH of the buffer (conditions as described in Figure 4).

Separation at low pH

In the method developed, the two compounds were separated at pH = 2.5 (Figure 2). The peak efficiency for the main peak was about 2.5×10^4 theoretical plates/meter. In order to assess the repeatability of the method, 10 consecutive injections of sample mixture were performed. This experiment was repeated three times. Calculated RSD values of migration times were found to be in the range of 0.8-1.3% for both compounds and the RSD values for peak areas were calculated 3.0-4.3% for aciclovir and 2.1-3.2% for guanine. The results and the RSD values for the last experiments are given in Table 1**.**

CZE method development at high pH

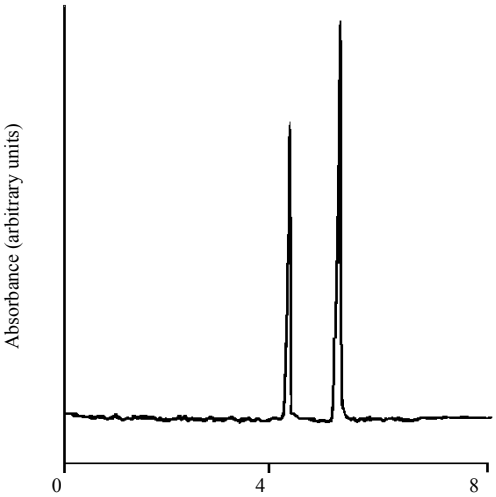

Figure 4. Separation of aciclovir and guanine under CZE conditions using a buffer of higher pH.Conditions: capillary 720 mm (500 nm to detector), buffer sodium tetraborate 20 mM, pH=9.8, voltage 25 kV, wavelength 254 nm, temperature(25°c)

Several operating parameters were considered in the method development procedures, including buffer pH and concentration, voltage and temperature. Preliminary experiments revealed that pH is the most important parameter which affects the resolution of aciclovir and guanine. The resolution of these compounds over a range of pH between 8 and 10 at two levels of buffer concentration is shown in Figure 3. The two compounds were separated satisfactorily at pH 9.5-10.0. Lower buffer concentration (i.e. 20 mM) was preferred because it produced lower current and therefore, lowers Joule heating. It would also be possible to increase the applied voltage, when dilute buffer was used. Therefore, the separation would be complete in a shorter time (Figure 4). The peak efficiency for the main peak was about 2×10^5 theoretical plates/meter.

Comparison of the two CZE methods

As shown the CZE method for determining aciclovir and guanine can be developed at two pH ranges. In terms of resolution, separation of the drugs in acidic buffer is more satisfactory, although the separation is achieved in a longer time but a higher voltage is applied. But the peak efficiency of the separation was obviously much higher in alkaline pH, due to increased electroosmotic mobilities. However, an essential aspect is reproducibility of results and the quantitative results must be repeatable over a series of experiments. In this case, the CZE method with the higher pH showed better RSD values. As a result the alkaline conditions were preferred for quantitative analysis of the drug aciclovir and its related component, guanine.

One possible explanation for the poor repeatability results in acidic buffer is that at low pH both compounds are positively charged, highly polar and possess some functional groups capable of making hydrogen bonds. Therefore, the possibility of capillary-wall interaction (i.e. adsorption onto the inner surface of the capillary) is more likely to occur at low pH, whilst, in the high pH range, both compounds are negatively charged and therefore, are repelled by the silanol groups at the inner capillary surface.

Table 1. The results of repeatability assessment of CZE method at pH=2.5.

	Aciclovir		Guanine	
No.	Time (min)	Peak area	Time (min)	Peak area
1	10.19	1465	5.53	911
2	10.23	1485	5.48	907
3	10.17	1503	5.53	921
4	10.18	1490	5.60	940
5	10.28	1541	5.49	946
6	10.21	1556	5.48	958
7	10.35	1577	5.55	949
8	10.41	1589	5.59	961
9	10.32	1583	5.62	958
10	10.39	1591	5.58	965
Mean	10.27	1538	5.54	942
SD	0.09	46	0.05	20
RSD%	0.83	2.99	0.85	2.12

Table 2. The results of repeatability assessment of CEZ method at pH=9.8.

	Aciclovir		Guanine	
No.	Time (min)	Peak area	Time (min)	Peak area
1	5.73	1005	6.13	1554
2	5.68	1004	6.11	1576
3	5.73	1043	6.10	1586
4	5.59	1003	6.12	1606
5	5.71	1016	6.17	1582
6	5.71	1005	6.15	1536
7	5.70	1038	6.14	1548
8	5.72	1037	6.16	1589
9	5.76	1040	6.15	1575
10	5.68	1025	6.14	1619
Mean	5.71	1022	6.14	1577
SD	0.02	16	0.02	24
RSD%	0.35	1.57	0.33	1.52

Validation of the CZE method at high pH

To show the reliability of the developed method, the CZE method at high pH was fully validated (11).

Repeatability: Ten consecutive injections of sample mixture were performed three times. The RSD values for migration times were 0.3-1.0%, and for peak areas were 1.5-2.6% for both compounds. The results and the RSD values for one series of experiments are given in Table 2.

Linearity assessment: The linearity of peak area for aciclovir was assessed over two ranges of sample concentration. In the first series, the drug concentration range was 0.01-0.07 mg/ml, covering 20 to 140% of the nominal concentration. In the next series, the assessment was carried out over a wider range of 0.05-0.3 mg/ml of aciclovir. The linearity of peak area for guanine was also assessed over a concentration range of 0.005-0.05 mg/ml. The results are given in Table 3.

Limit of detection: In order to assess the background signal, initially 10 consecutive replicates of buffer injection were made. The limit of detection (LOD) for aciclovir, on the basis of signal-to-noise-ratio of 3, was 1×10^{-4} mg/ml. The definition of the lower limit of quantitation (LOQ) was taken to be the signal equal to ten times of mean background signal (3×10^{-4} mg/ml). Similar results were found for guanine.

*Reproducibilty:*To assess the reproducibility of the method, six tablet extracts at a nominal concentration of 0.05 mg/ml were subjected to CZE in duplicate and the RSD of the mean peak areas was calculated (2.54%, 6).

Table 3. Summary of the results for linearity experiments for CZE method at pH=9.8.

	Aciclovir (± SD) (0.01-0.07 mg/ml)	Aciclovir (± SD) (0.05-0.30 mg/ml)	Guanine (± SD) (0.005-0.050 mg/ml)
n	7	6	6
r	0.998± 5.5 $\times10^{-4}$	0.998± 3.3 $\times10^{-4}$	0.9992± 4.2 $\times10^{-4}$
Y-intercept	1.30	0.82	0.53
Slope	2.007± 0.011	0.234± 0.007	1.063 ± 0.012

Recovery assessment: Three tablet extracts and a standard solution of aciclovir (which had undergone the extraction procedure) were analyzed with the CZE method and the concentrations were calculated from the calibration curve. The recovery of aciclovir from Zovirax tablets was 98.6-101.8% (w/w).

Robustness: Amongst several operating parameters, buffer pH was shown to have the largest effect on the quantitative results. The effect of sample injection time and temperature were negligible. The robustness of the method was also assessed after two months by different operators. In these experiments, five known standard solutions of aciclovir and guanine (both at 0.05 mg/ml) were assessed and the results showed no significant differences.

Measurement of the drug and impurity

Determination of the main drug, aciclovir, was based on the calibration curve in the latter case and the nominal concentration of the samples was 0.05 mg/ml. The measurement of the impurity, guanine, was also based on a separate calibration curve, as the compound was readily available. An alternative for using calibration curve in the latter case is to determine the area/area% of the guanine peak against aciclovir peak. However, peak area normalization is necessary for this method.

Measurement of guanine in the analytical samples

Three solutions of a tablet extract were prepared at a concentration of 0.2 mg/ml and each one was injected into the capillary in replicate at a 10 seconds injection time. No trace of guanine was observed in any of the samples. The method was successfully used in the assay of Zovirax tablets.

Conclusions

Of the two CZE methods developed for the separation of aciclovir and guanine, the method based on alkaline buffer (borate pH=9.8) showed more consistent results on repeatability assessment. For the acidic buffer the poorer quantitative results could be occuring as a result of capillary wall interactions with both components at low pH. To optimize the method, several operating parameters were considered; amongst these the buffer pH had the greatest effect. The method was then fully validated and successfully applied to the determination of the drug and its related substance in the Zovirax tablet preparation.

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