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# Validated capillary electrophoresis method for the simultaneous determination of formic acid and acetic acid in Cephalosporin drug substances using indirect UV detection

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## ABSTRACT

*A rapid and sensitive capillary electrophoresis (CE) method was developed for the determination of formic acid and acetic acid content in Cephalosporin drugs using a background electrolyte (solution containing 5 mM Potassium hydrogen phthalate and 0.25 mM cetyltrimethyl ammonium bromide as a flow modifier adjusted to pH 7.2) along with an applied electric field of 20kV for 0.4 min. A 40cm fused silica capillary was used as a stationary phase. Indirect UV detection mode was employed for the determination of analyte at a wavelength of 210nm. Linearity and accuracy were performed in the concentration range of 5-50 μg mL$^{-1}$ and 0.1-0.75 % w/w respectively for formic acid and 2-40 μg mL$^{-1}$ and 0.04-0.75 %w/w respectively for acetic acid. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness, robustness, and stability in analytical solution. The limit of quantification and detection for formic acid was 2.516 μg mL$^{-1}$ and 0.775 μg/mL respectively while for acetic acid was 1.820 μg mL$^{-1}$ and 0.546 μg mL$^{-1}$ respectively.*

**Key words:** Capillary Electrophoresis, Cephalosporin, Formic acid, and Acetic acid, Indirect UV detection.

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## INTRODUCTION

Formic acid and acetic acid are being used in the synthesis of Cephalosporin drugs and therefore the quantification of these acids is essential as they are harmful to the human body. Few articles were reported for the determination of formic acid and acetic acid using ion chromatography [1, 2, 3], gas chromatography [4, 5, 6], manometrically [7], volumetrically [8], acid-base titration [9], gas permeation flow injection analysis [10]. As formic and acetic acids are present in the bulk matrix, quantification becomes difficult on direct UV detection since small organic signals

will largely suppressed by the elution of the drug substances. Considering this, the attention was focussed on capillary electrophoresis using indirect UV detection. A rapid and more sensitive method on capillary electrophoresis has been developed for the quantification of formic acid and acetic acid at the trace level in the cephalosporine drug. This method is capable of quantifying formic acid and acetic acid in the range of 5-50 µg/mL and 2-40µg/mL respectively.

## MATERIALS AND METHODS

### Chemicals

Cephalosporin drugs were synthesized in maulana azad college, Aurangabad, India. Analytical reagent grade formic acid and acetic acid were obtained from S.D. Fine Chemicals Ltd, Mumbai, India. Analytical reagent grade potassium hydrogen phthalate was obtained from Sigma aldrich (Steinheim, Germany) and Cetyl tirmethyl ammonium bromide from Acros (New Jersey, USA). HPLC grade methanol was obtained from Merck Ltd, Mumbai, India). The water used for the study was obtained from a Milli-Q System (Millipore, Bedford MA, USA).

### Instrumentation

Capillary electrophoresis analyses were performed on a Capillary electrophoresis instrument (Hewlett Packard, USA) coupled with PDA detector and an automated vial replenishment system. The data acquisition and processing were done with HP chemstation software enabling us to obtain electropherogram with enhanced integrator and peak purity plot. The analysis was performed using an uncoated fused silica capillary of total length 48.5 cm, effective length 40 cm and 50µm internal diameter (SRi capillary). The capillary cassette temperature was maintained at 30°C using peltier cooling system. Indirect UV detection mode was used choosing the detection wavelength as 210 nm and reference wavelength as 360 nm. Sample solution and standard solution was injected using the hydrodynamic mode of injection and voltage was ramped to 20 kV in 0.4 min. The required buffer solution was prepared by dissolving 1.02 gm of potassium hydrogen phthalate and 91.12 mg of Cetyl trimethyl ammonium bromide in 100mL water, adjusted to pH 7.2 with dilute KOH. One part of this buffer solution was diluted with one part of methanol and eight parts of water was used as background electrolyte. Prior to use, the capillary was flushed with 1N NaOH followed by water for 10 min each using a pressure of 940 mbar.

### Sample Preparation

A solution was prepared containing 5 mg $mL^{-1}$ cephalosporine drug in water and methanol in the ratio of 1:1.

## RESULTS AND DISCUSSION

### Formic acid and acetic acid content

As formic acid and acetic acid are UV transparent compounds, indirect UV detection mode was selected for the determination. The use of indirect photometric detection in CE is similar to its use in ion chromatography (IC), with the transparent analyte replacing the probe co-ions in the electrolyte and creating a negative signal [11]. However, the operating principle of indirect photometric detection in CE differs markedly from those in IC. In IC stichiometric displacement of the probe by the analyte retained on the ion exchange stationary phase occurs, wereas in CE the Kohlraush regulating function determines the probe displacement by the analyte [12]. As a

consequence, the elution strength and optical properties of the probe are the key parameters in IC, but the probe's mobility and optical properties must be considered in CE. Correctly choosing an indirect detection probe and providing suitable buffering of the electrolyte can be a truly intriguing task. Choosing the probe is governed by consideration of its mobility, spectral properties and basic physicochemical properties. The mobility and concentration of the probe are crucial for the separation performance of the method because they influence peak shapes and efficiency. The absorptivity of the probe at the detection wavelength is a key parameter influencing the method sensitivity. In CE, the electrophoretic mobility of the co-ion relative to that of the analyte determines the shape of the migrating analyte zone as a result of electromigration dispersion [13]. This relationship applies to indirect photometric detection. When the mobility (μ) of the probe changes from a high value for chromate (μ= -88.1 x $10^{-9}$ m2/Vs) to intermediate for phthalate (μ= -41.2 x $10^{-9}$ $m^2$/Vs) and to a low value for benzoate (μ= -32 x $10^{-9}$ $m^2$/Vs), the peak shapes change in a very characteristic way; they are skewed away from the position in the electropherogram where the probe itself would migrate. This electromigration dispersion originates from the difference between the mobility and conductivity of the analyte zone and that of the surrounding electrolyte. To obtain the sharpest and most symmetrical analyte peak, and hence the best possible detection limit, the value of the electrophoretic mobility of the probe should match that of the analyte. The difference between the analyte concentration in the migration zone and the probe concentration in the electrolyte should be maximum. The relative mobility of acetate and formate ion is (μ= -33.8 x $10^{-9}$ m2/Vs) and (μ= -41.2 x $10^{-9}$ $m^2$/Vs) respectively which is very close to phthalate probe (μ= -41.2 x $10^{-9}$ $m^2$/Vs) as compared to Pyromellitate probe (μ= -55.1 x $10^{-9}$ $m^2$/Vs) [14]. Therefore Phthalate buffer is very convenient electrolyte having a very close mobility match with analyte of interest [15].

As the acetic acid and formic acid is present in a very trace quantity, the concentration of the analyte is very less in sample solution compared to the electrolyte probe concentration. Therefore the difference between the analyte and probe concentration is maximum [13]. On the basis of above discussion the detection relies on the use of UV active buffer component with the same charge as electrolyte (Phthalate buffer). Also the EOF modifier (Cetyl trimethyl ammonium bromide) with reversed polarity mode (Cathode at inlet side) was applied to ensure the movement of formate and acetate ions towards the detector. Capillary electrophoresis technique therefore was chosen to determine the quantity of formic acid and acetic acid content in Cephalosporin drugs. Experimentally determined LOQ and LOD for formic acid are 2.516 μg $mL^{-1}$ and 0.755 μg $mL^{-1}$ respectively while for acetic acid are 1.820 μg $mL^{-1}$ and 0.546 μg $mL^{-1}$ respectively.

## Method Validation

### Specificity

Sample spiked with formic acid and acetic acid was analysed as per method (Fig 1). Further, known related substances of Cephalosporin along with formic acid and acetic acid were spiked in the sample to check their interference if any with formic acid and acetic acid peak. The area of the analyte peak in the sample spiked with formic acid, acetic acid and other known related impurities remains same compared to the area in the sample spiked with formic acid and acetic acid indicating that there is no interference of Cephalosporin related substances with formic acid

and acetic acid peak proving that the method is specific for the determination of formic acid and acetic acid in Cephalosporine drug substances.

From the above study we can conclude that, unless and until the charge to mass ratio of any other impurity is similar to that of analyte, it will not appear at the migration time window of the analyte. Therefore, on the basis of this study it is concluded that the peak is homogeneous and pure.

**Precision**

System precision was determined by injecting replicate standard solution containing 25µg/mL, 10µg/mL, and 5µg/mL each of formic acid and acetic acid into the CE system. The % RSD values of < 2 for corrected peak area counts and < 1 for migration time, indicates an acceptable level of precision for the analytical system.

Method precision was determined by analyzing six samples of single batch of cephalosporin as per proposed method. The formic acid content were determined from the ratio of response of corrected area counts in the sample and slope of calibration curve prepared from formic acid standard solutions of 5 µg/mL, 10 µg/mL, 25 µg/mL. The RSD value of 2.45 % for formic acid content and 2.93 % for acetic acid content indicate that the method has an acceptable level of precision.

Intermediate precision was determined by analyzing six samples of a single batch of cephalosporin by different analyst on a different day using a different capillary. The RSD value of 3.85% for formic acid content and 5.87 % for acetic acid content were obtained.

Ruggedness of the method was proved by the insignificant difference between the two sets of data (method precision and intermediate precision) as determined by the overall RSD value of 3.46 % for formic acid content and 7.33 % for acetic acid content.

**Linearity**

The linearity of response for formic acid and acetic acid was determined by injecting formic acid standard solution in duplicate in the range of about 5 – 50 µg $mL^{-1}$ and acetic acid standard solution in the range of about 2 – 40 µg $mL^{-1}$. The detector response for formic acid and acetic acid was found to be linear over the specified range as determined from the correlation coefficient value of 0.99935 and 0.99991 for formic acid and acetic acid respectively. The equation representing the calibration curve for formic acid was y = 0.00853 x –0.00034 and for acetic acid was y = 0.01134 x +0.00034.

**Accuracy**

A known amount of formic acid and acetic acid was added to cephalosporin sample solution at five concentration levels i.e; about 0.1, 0.2, 0.4, 5, and 0.75 % w/w (with respect to cephalosporin) in triplicate at each level. The samples were analyzed as per proposed method. Mean recovery of 96.30 % with RSD of 3.19 % and individual recovery values obtained in the range of 90-110%. indicates an acceptable level of accuracy for the proposed method.

**Sensitivity**

The Limit of detection and quantification for formic acid and acetic acid was determined from linearity data and verifying the predicted LOD and LOQ values by showing precision at these concentrations. Experimentally determined LOQ and LOD for formic acid are 2.516 µg/mL and 0.755 µg/mL respectively while for acetic acid are 1.820 µg/mL and 0.546 µg/mL respectively.

**Stability in analytical solution**

A sample solution spiked with formic acid and acetic acid was prepared and kept at ambient room temperature. The sample solution was analyzed initially and at regular time intervals. The sample was found to be stable for about 7 hours at room temperature

**Robustness**

The Robustness of the method for formic acid and acetic acid was verified by analyzing a sample of cephalosporin by varying capillary cassette temperature (± 5°C), BGE pH (± 0.5) and voltage (±2kV). The robustness of the method is indicated by the insignificant difference between the two sets of data (Control and variable conditions) as determined by overall RSD values, which are always less than 10% indicates that the method is robust towards temperature, voltage and voltage.

**Replenishment frequency test**

Standard solution of formic acid and acetic acid (10 µg $mL^{-1}$ each) was injected 30 times into the CE system using a set of background electrolyte .The RSD value indicates that background electrolyte should be replenished after every 13 injections as determined by the % deviation of less than 5% for 13 consecutive injections (% deviation calculated from the mean of first six injections).

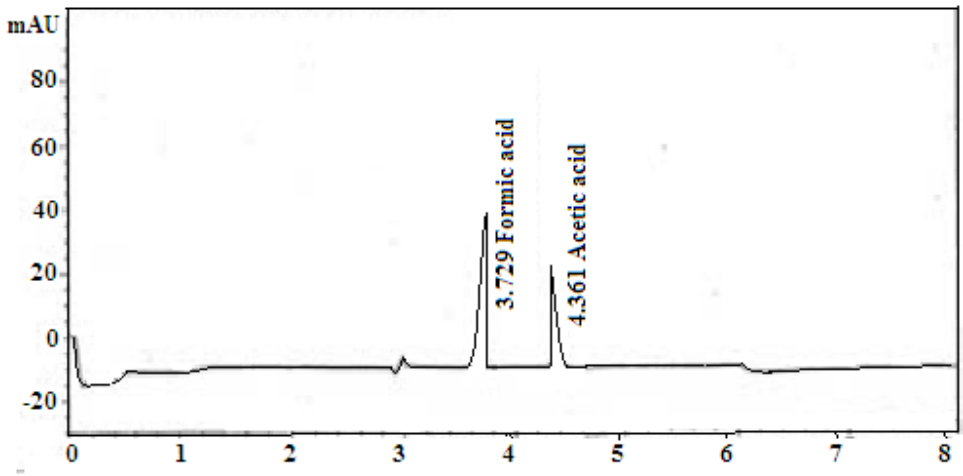


**Figure 1: Electrophorogram of Sample solution spiked with formic acid and acetic acid**

## CONCLUSION

Indirect photometric detection is a useful detection technique for nonabsorbing analytes. For an indirect photometric detection method to perform optimally, the analyst must consider several key parameters, including peak shape, peak efficiency, detection sensitivity, baseline noise, and buffering of electrolyte to ensure method ruggedness. These principles impose a number of criteria in designing a suitable indirect photometric detection method. First, the probe should be chosen for closeness of its mobility to the mobilities of critical analytes, and it should provide the maximum absorbance change when displaced by an analyte ion. Second, co-ionic impurities in the electrolyte should be avoided because these cause system peaks and decrease in detection sensitivity and they always contribute to unstable baseline. Considering all the above parameters, a rapid and simple CE method for the simultaneous determination of formic acid and acetic acid has been developed. This CE method provides excellent reproducibility, good linearity, accuracy and appropriate sensitivity. These results indicate that the proposed method is robust and rugged and can be useful for routine analysis of formic acid and acetic acid in any cephalosporin drug.

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## REFERENCES

[1] Y. Suzuki, *Anal. Chim. Acta*, 353 (**1997**) 227-237.
**[2]** V. Magne, M. Mathlouthi, B. Robilland, *Food Chem*. 61 (**1998**) 449-453.
[3] D.A. Guillen, C.G. Barroso, L. Zorro, V. Carrascal, J.A. Perez-Bustamante, *Analysis* 26 (1998) 186-189.
[4] G. Wittman, H. Van Langenhov, J. Dewulf, *J.chromatogr A* , 874(**2000**), 225-234
[5] S.A.Kupina, J. L. Kutschinski, R.D. Williams, R.T.deSoto, A *Am.J.Enol.Vitic*, 33(**1982**), 67-74
[6] P. Cvijin, D. Vujkovic, G. Atkinson, *Applied spectroscopy*, 42, (**1988**), 770-774
[7] M. Pickett, H. Ley and N. S. Zygmuntowicz, Monometric determination of formic acid (1944) 303-315.
[8] Chi Hung Tazang, Ruo Yuna and Mengsu Yang, *J. Biosensors and Bioelectronics*,16, (**2001**), 211-219.
[9] Narong Lenghor, Jaroon Jakmunee, Michael Vilen, Rolf Sara, Gary D.Christian and Kate Grudpan, *J. Talanta*, 58, (**2002**), 1139-1144.
[10] C. TavatesnAraujo, J. Lira de Carvalho, D. Ribeiro Mota,C.L.de Araujoand N.M.M. Coelho, *J. food chemistry*, 92, (**2005**), 765-770
[11] P. Haddad, P. Jackson, Calippary electrophoresis, Elsevier, Amsterdam, (**1990**).
[12] F. Foret, L. Krivankova, P. Bocek, Capillary zone electrophoresis, VCH Publishers, (**1993**), 27-35.
[13] C. Johns, M. Macka, P. Haddad, *Electrophoresis*, 23, (**2002**), 43-48
[14] K.D. Altria, Indirect Photometric Detection in CE Using Buffered Electrolytes — Part II, Practical RulesLCGC, 19 (**2001**) 178-188.
[15] Z. Chen, B.Gold, M. Adams, *J.Chrom A*, 818 (**1998**) 61-68.