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Separation of imidazole and its derivatives by capillary electrophoresis ☆

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Abstract

The use of capillary electrophoresis (CE) for the separation of imidazole and its derivatives is reported. In the first part of the investigation, efforts were focused on method development, where the effects of buffer pH, types of buffer modifiers and modifier concentration on the separation of these compounds were examined. The second part was focused on method validation, where the ruggedness, selectivity, limits of detection and linearity were investigated. In the final part, the CE method was applied to commercial-grade imidazole. A comparison of the results obtained using the CE system was made with those obtained by HPLC. Good correlation between the two sets of results was obtained and superior efficiencies and better peak shapes for most of the imidazoles were also achieved using the CE system.

1. Introduction

The use of capillary electrophoresis (CE) in separation science has increased tremendously in recent years. Major attributes of CE are the high efficiencies and separation selectivities possible [1,2]. As a result, separations of mixtures which in the past have been classified as inseparable or very difficult to chromatograph may nowadays be possible using CE. Consequently, there is growing interest in its use as a supplement to or as a replacement for existing methods such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) [1].

In this work, the use of CE for the separation of imidazole and its derivatives is demonstrated. The imidazole ring is a component of several important groups of compounds such as purine, histamine and histidine. As a result, imidazoles are widely used as starting materials in the pharmaceutical industry [3] and as important intermediates in herbicides [4]. Therefore, the purity of imidazole and its derivatives used as starting materials is an important aspect to consider in industrial processes.

Currently, most imidazoles are analysed using techniques such as HPLC or GC. HPLC, even though routinely used, suffers from the usual peak tailing commonly observed in the analysis of basic compounds. GC analysis is limited to only a few volatile imidazoles. Therefore, in this work, attempts were made to study the feasibility of using CE for the separation of imidazoles.

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As part of method development, the effects of buffer pH, modifiers and modifier concentrations on the separation of these compounds were examined. The migration behaviour of these compounds under the various experimental conditions was investigated. A key focus of this work was an attempt to perform method validation on the CE system. In this regard, the ruggedness, selectivity, limits of detection and linearity range of the CE system were examined. The methodology developed was demonstrated using laboratory-built and commercial instruments. Further, the results obtained were compared with those obtained by HPLC.

3. Experimental

3.1. Instrumental

Experiments were conducted on a laboratory-built CE system and two commercial instruments. In the laboratory-built instrument, a Spellman (Plainview, NY, USA) RM15P10KD power supply capable of delivering up to 15 kV was used. A Carlo Erba (Milan, Italy) MicroUVis20 UV detector with the wavelength set at 214 nm was used for the detection of peaks. A P/ACE 2000 (Beckman, Palo Alto, CA, USA) and a Waters Quanta 4000 (Milford, MA, USA) were on loan from the respective instrument companies. UV filters of 214 nm were used for both commercial instruments. Untreated fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). Chromatographic data were collected and analysed using a Hewlett-Packard (Avondale, Palo Alto, CA, USA) HP3394A integrator and a Shimadzu (Kyoto, Japan) R-6A integrator.

3.2. Chemicals and materials

Sodium dodecyl sulphate (SDS), sodium tetraborate and sodium dihydrogenphosphate were purchased from Fluka (Buchs, Switzerland), tetrabutylammonium hydrogensulphate (TBA) from Sigma (St. Louis, MO, USA) and 1-methylimidazole and 4-methylimidazole from Aldrich (Milwaukee, WI, USA). Standards of imidazole and 2-methylimidazole of analytical-reagent grade were gifts from Glaxo Development (Singapore). Samples of 2-methylimidazole used for the assay and impurity studies were also supplied by Glaxo Development.

3.3. Procedures

Standards at a concentration of 1 mg/ml were dissolved in HPLC-grade methanol. SDS and TBA of various concentrations were dissolved in 25 m*M* sodium tetraborate–sodium dihydrogenphosphate buffers.

4. Results and discussion

4.1. Method development

Effect of pH

Initial experiments performed using capillary zone electrophoresis (CZE) conditions at various pHs showed some interesting trends. At pH < 8, two peaks corresponding to the four imidazoles which eluted before methanol were noted. On the other hand, a single peak was observed at pH >8.

This observation is consistent with the fact that the imidazoles are moderately strong bases. As a result, positive charges may reside on the imidazole ring under suitable pH conditions (in this case, pH <8). As in typical CZE separations, these positively charged species would tend to migrate out earlier than the neutral solvent molecules. However, at higher pH (>8), these compounds are neutral. As selectivity in CZE tends to be poor for the separation of neutral compounds, it was therefore expected that at pH >8 the neutral imidazoles would co-elute with methanol.

As the imidazole derivatives are very similar in structure, optimization of separation by varying the pH alone would not be sufficient. In CE separations of neutral species, modifiers such as surfactants [5] and tetrabutylammonium salts are usually added to enhance selectivity. In this

work, SDS and TBA as modifiers were investigated.

Effect of sodium dodecyl sulphate (SDS)

Fig. 1 shows the migration behaviour of the imidazole derivatives at various SDS concentrations. A general trend observed is that with the addition of SDS to the buffer system, e.g. at 60 m*M*, almost complete separation of the imidazoles was obtained. This marked improvement in the selectivity is possible as the addition of SDS is expected to provide a micellar environment which acts as a pseudo-stationary phase for the system [5]. The neutral imidazole molecules, in response to the micellar environment, are expected to undergo interaction with SDS micelles via solubilization into the cavity of the SDS micelles. The extent of interaction is expected to be dependent on the hydrophobicity of the imidazoles. The more hydrophobic, i.e., the more alkylated, the imidazole is, the more it would favour solubilization and therefore, it would migrate out later. In this way, different imidazoles would be separated as a result of the enhancement in selectivity due to the addition of SDS.

Another trend observed is that with increasing amount of SDS in the system, a corresponding increase in migration times for the imidazole was noted. It should be recognized that the increase in concentration of SDS in the buffer would promote and increase the probability of the interaction of the neutral imidazole with the micellar environment. As the negatively charged micelles are electrophoretically attracted to the anode, this would subsequently lead to a corresponding increase in migration times of the imidazoles.

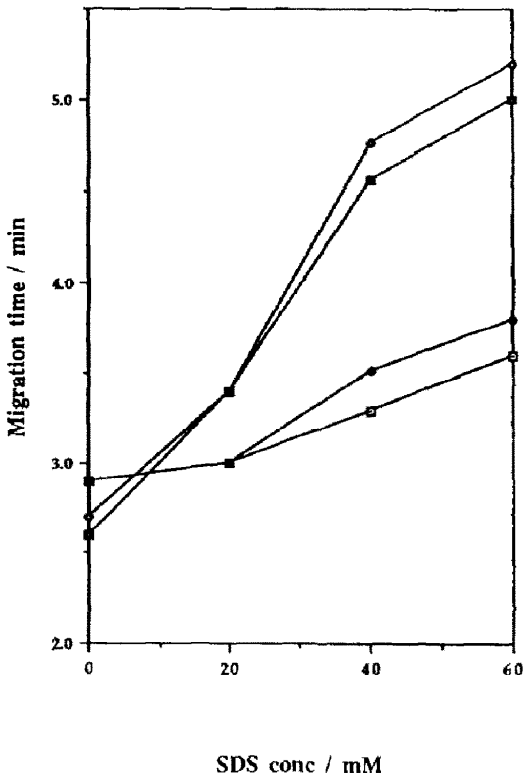

Fig. 1. Plot of migration times versus SDS concentration. Electrophoretic conditions: 25 m*M* phosphate–borate buffer (pH 8) with various SDS concentrations; voltage, 15 kV; column, 40 cm effective length × 50 μm I.D. fused silica; detection wavelength, 214 nm. □ = Imidazole; ◆ = 1-methylimidazole; ■ = 2-methylimidazole; ◇ = 4(5)-methylimidazole.

Effect of tetrabutylammonium hydrogensulphate (TBA)

Fig. 2 shows the optimum chromatogram obtained with the addition of 10 m*M* TBA to the electrolyte solution. It can be seen that a signifi-

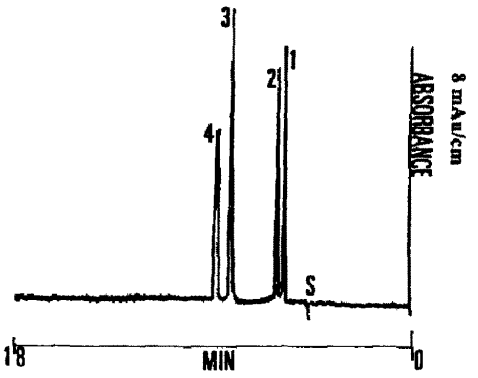

Fig. 2. Typical electropherogram for the separation of the imidazole derivatives. Electrophoretic conditions: 25 m*M* phosphate–borate buffer (pH 8.7)–100 m*M* SDS–10 m*M* TBA; voltage, 15 kV; column, 40 cm effective length × 50 μm I.D. fused silica; detection wavelength, 214 nm. Peaks: S = methanol; 1 = imidazole; 2 = 1-methylimidazole; 3 = 2-methylimidazole; 4 = 4(5)-methylimidazole.

cant improvement in the peak shape was achieved using this system. This observation is consistent with those observed by Ong et al. [6] and Nishi et al. [7], i.e., the addition of TBA not only provided an additional pseudo-stationary phase to enhance selectivity, but also improved the overall peak shape of the positively charged imidazole derivatives by decreasing the interaction of these compounds with the negative charges on the capillary wall.

4.2. Method validation

Selectivity

From Fig. 2, it can be seen that all the peaks were completely separated from each other with a resolution of at least 1.5. It should be emphasized that even though most of the imidazole derivatives differ from each other merely by the difference in the attachment of the methyl group to the main imidazole structure, the CE system developed in this work was able to provide the selectivity required for separation. In addition, the reduction in peak tailing and improvement in selectivity synergistically improved the overall separation and quantification capability. As a result, the method would provide an accurate identification and quantification method for imidazole and its derivatives. Further, it is worth noting that the total analysis time required was less than 10 min. This is a very attractive feature for implementation as a routine analytical method.

Ruggedness

The results obtained so far were obtained using the laboratory-built system. To demonstrate the ruggedness of the method, the same separation was performed on two commercial CE systems. Figs. 3 and 4 show the results obtained using the Beckman and Waters CE systems. Similar separation profiles with all three systems are observed (Figs. 2–4). The slight differences in migration times and detection responses observed were attributed to the differences in column lengths and detection windows. Nevertheless, from these results, it can be clearly seen that the CE method developed is a very

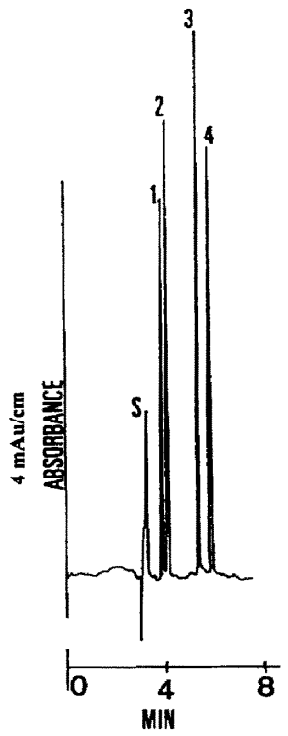

Fig. 3. Typical electropherogram for the separation of the imidazole derivatives using the Beckman commercial CE system. Electrophoretic conditions and peak identification as in Fig. 2.

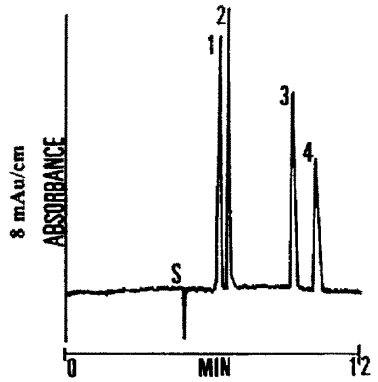

Fig. 4. Typical electropherogram of the separation of the imidazole derivatives using the Waters commercial CE system. Electrophoretic conditions and peak identification as in Fig. 2.

Table 1
Comparison of the assay of 2-methylimidazole using HPLC and CE with commercial instruments

HPLC		Beckman		Waters	
Purity (%)	R.S.D. (%) ($n = 3$)	Purity (%)	R.S.D. (%) ($n = 3$)	Purity (%)	R.S.D. (%) ($n = 4$)
100.2	0.2	100.0	0.25	99.5	3.0

robust technique that is independent of instrumentation as long as similar parameters are used.

Linearity and detection limits

A linear calibration graph was obtained for the 2-methylimidazole at concentrations up to 1 mg/ml. Excellent linearity was observed with correlation coefficients greater than 0.999. A detection limit at ca. 30 pg using UV detection at 214 nm was obtained. These results are within the regular working range suitable for the analysis of these compounds.

4.3. Application

The method was successfully employed to analyse samples obtained commercially. The results are given in Table 1. A typical HPLC trace and an electropherogram obtained using the Beckman instrument are shown in Figs. 5 and 6, respectively. A very important observation is that the extent of peak tailing in the CE analysis is much less than that in HPLC (Fig. 5). From Table 1, it can be seen that a good correlation was obtained for the three sets of results. The use of an internal standard, 4-methylimidazole, and more efficient temperature control resulted in better reproducibility in the CE analysis using the Beckman instrument compared with the Waters system. Although the R.S.D. for the analysis using the Waters instrument was slightly higher than those obtained by HPLC and the Beckman instrument, the results correlate very well with each other.

5. Conclusions

CE is a viable alternative method for the separation of imidazole and its derivatives. Method validation was successfully performed to

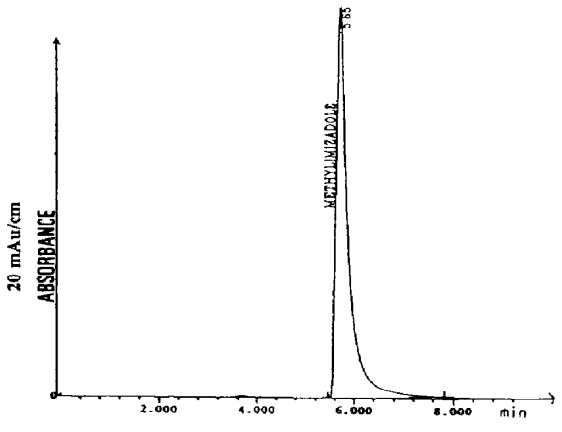

Fig. 5. Typical chromatogram for a commercial sample obtained using HPLC.

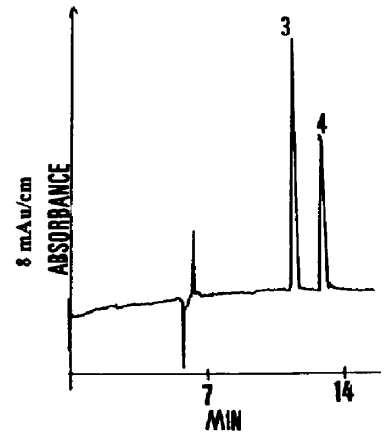

Fig. 6. Typical electropherogram for a commercial sample obtained using the Beckman commercial CE system. Electrophoretic conditions and peak identification as in Fig. 2.

confirm the ruggedness, selectivity and detection limits of the method. When comparing the results with those of HPLC, a good correlation was obtained between the two techniques. Further, CE exhibits less peak tailing and hence is preferable in terms of quantification and selectivity. The relatively short analysis time required for the analysis is another feature of the technique that is especially attractive for implementation in routine analysis.

Acknowledgements

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