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Separation and identification of the *Z* and *E* isomers of 2-(3-pentenyl)pyridine by capillary electrophoresis and nuclear magnetic resonance spectroscopy

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Abstract

A mixture of the *Z* and *E* isomers of 2-(3-pentenyl)pyridine has been separated with baseline resolution by capillary electrophoresis. Using molecular modelling it was proposed that the smaller more rapidly migrating peak would be the *Z* isomer. This agreed with a 38:62 (*Z*/*E*) composition by nuclear magnetic resonance spectroscopy. The sample was also investigated by gas chromatography coupled to mass spectrometry.

1. Introduction

Capillary electrophoresis (CE) is rapidly developing as a complementary technique to high-performance liquid chromatography (HPLC) for the separation of small molecules [1]. Since CE is based on different physicochemical properties than HPLC, different performance characteristics are obtained, such as higher efficiencies and rapid times of separation. Together with low consumption of buffer and small sample requirements, it is clear that CE is an attractive method of separation for the pharmaceutical industry.

Separation in CE depends on the movement of the analyte ions in the applied electrical field. The electrophoretic mobility, μ, of a particle is defined as the steady state velocity per unit field strength, $\mu = q/f$, where q is the charge on the analyte and f is the frictional coefficient of the analyte. Thus, the principal parameters that can influence separation are the charge on the analyte, q, and the factors which influence the frictional coefficient, f, which are the size of the analyte and also the shape of the analyte ion.

Rowe et al. [2] have investigated the influence of size/shape on the separation of the monosubstituted alkyl pyridines. The separation of positional isomers was achieved and various molecular descriptors were investigated to predict the mobility of the analytes. Chadwick and Hsieh [3] have also reported the separation of the alkenes, fumaric acid, maleic acid, all *trans*-retinoic acid and 13-*cis*-retinoic acid. Differences in mobility were ascribed to differences in shape of the alkenes, which were regarded as spheres with different hydrodynamic radii.

Quantitative impurity content determination by CE has been shown to be of comparable precision to HPLC by Altria [4,5]. It was shown that the analytes must have the same UV chro-

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mophores or correction factors applied, and that peak areas must be normalised by migration time in order to correct for the effect of analytes spending different residence times in the portion of the capillary that acts as the detection cell, because of the different mobilities.

In this study a mixture of the *Z* and *E* isomers of 2-(3-pentenyl)pyridine were investigated as model compounds, and the relative abundance of each isomer was determined by CE and compared to the nuclear magnetic resonance (NMR) spectrum of the sample. The sample of 2-(3-pentenyl)pyridine was also investigated by gas chromatography coupled to mass spectrometry (GC–MS).

2. Experimental

2.1. Chemicals

The sample of 2-(3-pentenyl)pyridine was used as received from Aldrich (Poole, UK) and was a mixture of the *Z* and *E* isomers. ^{1}H NMR spectra were run in deuterated chloroform on a 250 MHz AC250 Brüker NMR spectrometer. The sample of 2-(3-pentenyl)pyridine as a dilute solution in dichloromethane was also analysed using GC–MS on a Fisons GC 8000 gas chromatograph coupled to MD 800 mass spectrometer using a DB 5 ms column isothermally at 100°C. Molecular modelling measurements were made using Nemesis Sampler (Oxford Molecular).

2.2. Capillary electrophoresis

Work was carried out on a P/ACE 2050 system (Beckman Instruments, High Wycombe, UK) using a fused-silica capillary of 57 cm (50 cm to detector) × 75 μm I.D. Samples were loaded by a 2-s pressure injection at the anode and separated using a voltage of 15 kV. The external temperature of the capillary was thermostated at 25°C. The capillary was rinsed between each injection with a rinse cycle of sodium hydroxide (0.1 *M*, 2 min) and running buffer (3 mins). The peaks were detected at 254 nm using a 2 Hz collection rate. Electrophoretic separations were performed in a phosphate buffer (40 m*M*) prepared from orthophosphoric acid (BDH, Poole, UK) and adjusted to pH 2.5 with lithium hydroxide (1 *M*; FSA, Loughborough, UK).

3. Results and discussion

2-(3-Pentenyl)pyridine is marketed as a mixture of isomers and in a solution at pH of 2.5 these can readily be separated by CE in 11 mins (Fig. 1). Little method development was required, as 2-(3-pentenyl)pyridine was fully protonated under these conditions. However, it was not possible to directly assign structures to the peaks in the electropherogram but as electrophoretic mobility increases with decreasing size of the analyte it should be possible to use molecular modelling to predict the migration order of the isomers. When the lengths of the side chains were calculated, it was found from a molecular modelling package that the *E* isomer was fully extended with a length from the *ortho*-hydrogen on the pyridine ring to the end of the sidechain of 10.2 Å. The corresponding measurement for the *Z* isomer was 9.2 Å (Fig. 2). Thus the smaller peaks at 10.11 min was predicted to correspond to the *Z* isomer and the second larger peak at 10.31 min to the *E* isomer. These assignments agreed with the expected ratio of the isomers based on the greater thermodynamic stability of the *E* isomer.

In order to quantify the proportion of the two isomers, the absorbance of the analytes are required. Because of the similarity of the chromophore it was expected that the spectra would be effectively identical and this was confirmed using a CE system equipped with a diode array detector. Both peaks gave identical spectra with a maximum absorbance at 265 nm. It was also necessary to correct the peak areas by division by their migration times, in order to take account of the unequal residence time of the analytes in the detection window. Using this correction, the mean proportions over six runs was 40.9% for the minor isomer and 59.1% for

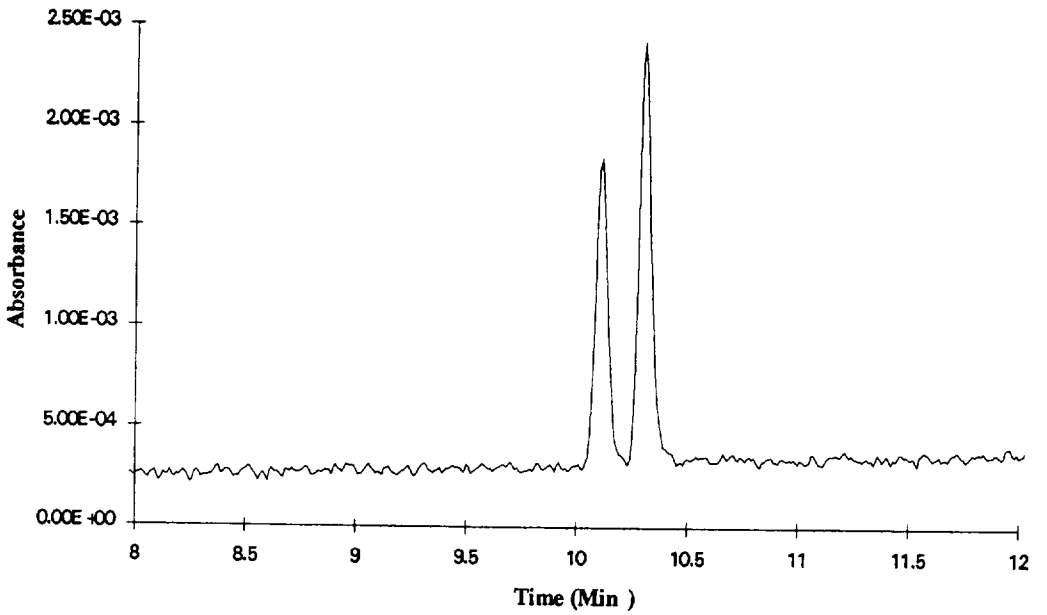

Fig. 1. Electropherogram showing the separation of (*Z*)- and (*E*)-2-(3-pentenyl)pyridine.

the major isomer with a standard deviation of 0.9%. The precision of these measurements is in agreement with the work of Altria [5], who reported precisions of 1–2% relative standard deviations.

Confirmation of the assignment of the isomers can be obtained by using 1H NMR spectroscopy to independently determine the proportion of the isomers. The shifts of the terminal methyl groups adjacent to the double bonds are different for the *Z* and *E* isomers, and each are split due to the adjacent protons. Coupling across the double bond was also observed, which split each methyl signal into a doublet of doublets and can be used to identify the isomers (Fig. 3). The larger peaks showed a long-range coupling of 1.1 Hz and were therefore assigned to the *E* isomer, whereas the small peaks had a coupling of 0.6 Mz typical of a *Z* isomer. It was also predicted that the methyl group in the *Z* isomer would be more shielded than in the *E* isomer, thus it would occur at a higher field as was observed. The heights of the two methyl signals were compared to give relative concentrations of each isomer in the sample, giving a percentage of (*Z*)-2-(3-pentenyl)pyridine of 38% and (*E*)-2-(3-pentenyl)pyridine of 62% in agreement with the CE assignments.

In order to ensure that the two peaks were isomers and not homologues or other derivatives a sample of 2-(3-pentenyl)pyridine was also investigated by GC–MS. At 100°C under isothermal conditions, major peaks were obtained at 16.0 and 17.1 mins, which had identical mass spectra with molecular ions (*m/z* 147), which correspond to 2-(3-pentenyl)pyridines. A minor impurity was also present. The proportions of the major peaks differed from those observed by the other techniques which was attributed to thermal equilibration of the isomers.

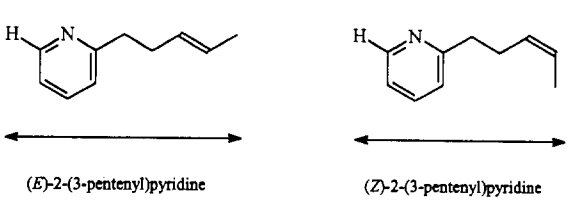

Fig. 2. Structures of (*Z*)- and (*E*)-2-(3-pentenyl)pyridine.

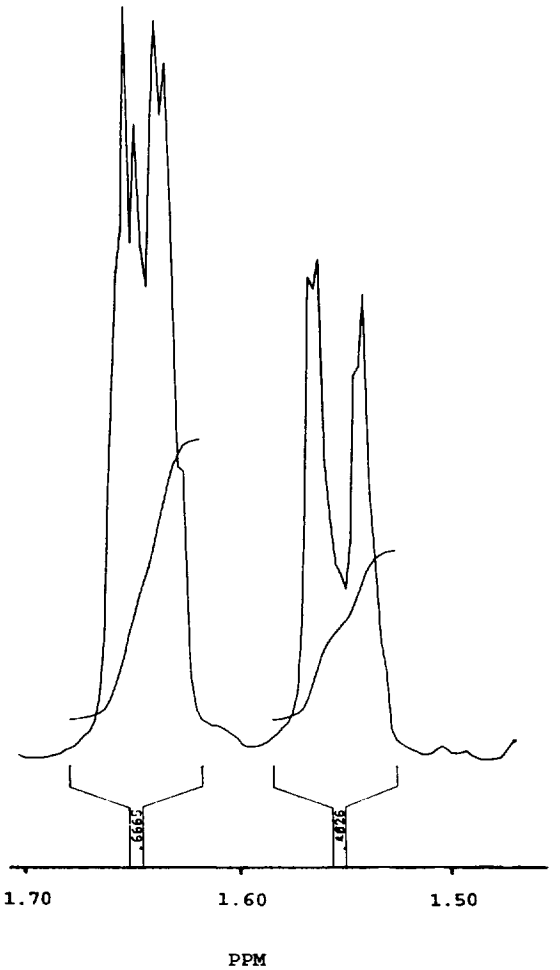

Fig. 3. Expanded methyl signals in the NMR spectrum of 2-(3-pentenyl)pyridine.

4. Conclusions

The *Z* and *E* isomers of 2-(3-pentenyl)pyridine can be separated according to their shape by CE. By considering the shape of the molecules, it was possible to predict the order of migration which was subsequently confirmed by NMR spectroscopy.

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