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Open access capillary electrophoresis A walk up capillary electrophoresis service for the synthetic chemist☆

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Abstract

A capillary electrophoresis (CE) system has been developed to provide routine purity information to support synthetic organic chemists. A single micellar electrokinetic chromatography system that produces good selectivity with short run times was developed. The instrument operating software has been modified to run separations in a custom open access mode. No expert knowledge of CE is required to run separations. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Synthetic organic chemists engaged in pharmaceutical research require a range of analytical and spectroscopic techniques to support their work.

Analytical separation techniques available to chemists include thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). These techniques have some limitations: TLC often lacks the necessary selectivity for the separation while HPLC runs can be long and a thorough knowledge of system operation is needed.

These approaches are not the only ones available to chemists. Capillary electrophoresis (CE) has emerged as a complementary separation tool to HPLC [1–3]. This is a powerful method of separating compounds on the basis of charge, size and, with the right choice of buffer additive, hydrophobicity. It is a relatively easy technique to understand, and the operating parameters are more easily optimised than in TLC or HPLC. An expert knowledge of running analytical instruments is not necessary as open access software can be developed for data collection. Open access systems have been successfully applied in mass spectrometry (MS) [4,5], nuclear magnetic resonance (NMR) [6], liquid chromatography (LC)–MS [7] and gas chromatography (GC)–MS [8]. Thus, CE appears to be a promising technique for creating a truly "open access" (OA) approach for the synthetic chemist.

1.1. Micellar electrokinetic chromatography theory

Free solution CE enables the separation of compounds on the basis of charge and size, however to satisfy the system requirements for a generic method

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capable of separating a broad range of charged and neutral compounds a micellar method is required. Micellar electrokinetic chromatography (MEKC) is a technique introduced by Terabe et al. in 1984 [9]. It is essentially a hybrid of electrophoresis and chromatography. It has become one of the most popular CE modes as it allows the separation of neutral as well as charged molecules. Separation in MEKC is achieved by adding a surfactant to the buffer. When added above the critical micelle concentration the surfactants form aggregates called micelles. Sodium dodecyl sulphate (SDS) is an anionic surfactant, forming micelles at concentrations above 9 m*M*. The characteristics of the spherical aggregate produced by anionic SDS micelles are hydrophobic aliphatic chains in the interior with hydrophilic sulphate heads on the exterior orientated towards the aqueous buffer solution. During electrophoresis the SDS micelles migrate towards the anode. Operating at neutral or basic pH conditions ensures that the electroosmotic flow (EOF) is faster than the micelle migration velocity, resulting in a net micellar movement in the direction of the EOF. During electrophoresis analytes can hydrophobically and electrostatically interact with SDS micelles in a chromatographic manner. For neutral analytes, partitioning in and out of micelles is the key to achieving separations. When a neutral analyte resides in the aqueous solution it moves with the EOF. However, when partitioned into a micelle it is carried against the EOF. As a result the more hydrophobic the analyte, the longer it will reside in SDS micelles and the longer the resulting elution time.

The elution time window for neutral analytes is between t_0 and t_m. Hydrophilic analytes which do not interact with the micelles are eluted at t_0, whilst analytes totally retained by the micelles are eluted with the micelles at t_m. A key requirement for MEKC is to optimise this elution time window to meet the requirements of the method. This elution time window can be changed by varying the buffer pH, surfactant concentration, buffer concentration, temperature, or via the addition of organic solvents, urea or chiral selectors. Using basic pH conditions promotes ionisation of the silica surface in the capillary and results in a faster EOF than under lower pH conditions. Increasing the surfactant concentration increases the elution time window and can improve the selectivity of the method. Increasing the buffer concentration gives a less pronounced increase in the elution time window although it can improve peak shapes as a result of the focusing effect known as stacking [10–12]. To develop a viable OA method a balance between speed and selectivity is required. By careful selection of three key parameters; pH, surfactant concentration and buffer concentration a short, selective method capable of resolving neutrals, acids and bases may be attainable.

2. Experimental

2.1. Chemicals

Sodium borate (Borax) and SDS were both obtained from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile was obtained from Romil (Cambridge, UK). Quinine, cinchonine, indapamide, tryptophan, bendroflumethiazide and camphor-*p*-tosylhydrazone were obtained from Sigma. The tioconazole precursor regioisomers and hydrogenation reaction mixture samples were obtained from Pfizer (Sandwich, UK).

2.2. Conditions

Experimental investigations were carried out on a Hewlett-Packard ^{3D}CE/CEC instrument (Berkshire, UK). Detection wavelengths of 195 nm, 200 nm, 220 nm, 234 nm and 254 nm were used for all samples. The separations were performed in a fused-silica capillary purchased from Composite Metal Services (Worcester, UK) with dimensions 48.5 cm (40 cm to the detector)×75 μm I.D. The micellar run buffer used was 20 m*M* borax, 18 m*M* SDS, pH 9.2.The capillary was air cooled to 25°C. Samples were kept at ambient temperature and injected via applying 50 mbar pressure for 2 s. Each new capillary was preconditioned with a 1 *M* sodium hydroxide wash for 30 min, followed by a 0.1 *M* sodium hydroxide rinse for 30 min and then a 30 min rinse with buffer. Between analyses capillaries were flushed with 0.1 *M* sodium hydroxide for 30 s and then with run buffer for 2 min. Sample handling needs to be simple and compatible for analysis using aqueous micellar conditions. Chemists can take a few μl of the

solution used to obtain an NMR spectrum of their product and dilute this to 200 μl with water or a CE-compatible solvent. NMR sample solutions in dimethyl sulphoxide (DMSO), MeO^2H, ^2H_2O are directly miscible with CE-compatible buffers; however, C^2HCl_3 solutions are dried down and redissolved in a CE-compatible solvent. Alternatively, the chemist can take as little as 0.1 mg or as much as 1 mg of solid or gum and dissolve in water or CE compatible solvent. The system is protected from samples which contain insoluble particles by passing each solution through a 0.45-μm filter.

2.3. Practical investigations

Compounds selected to test the effectiveness of

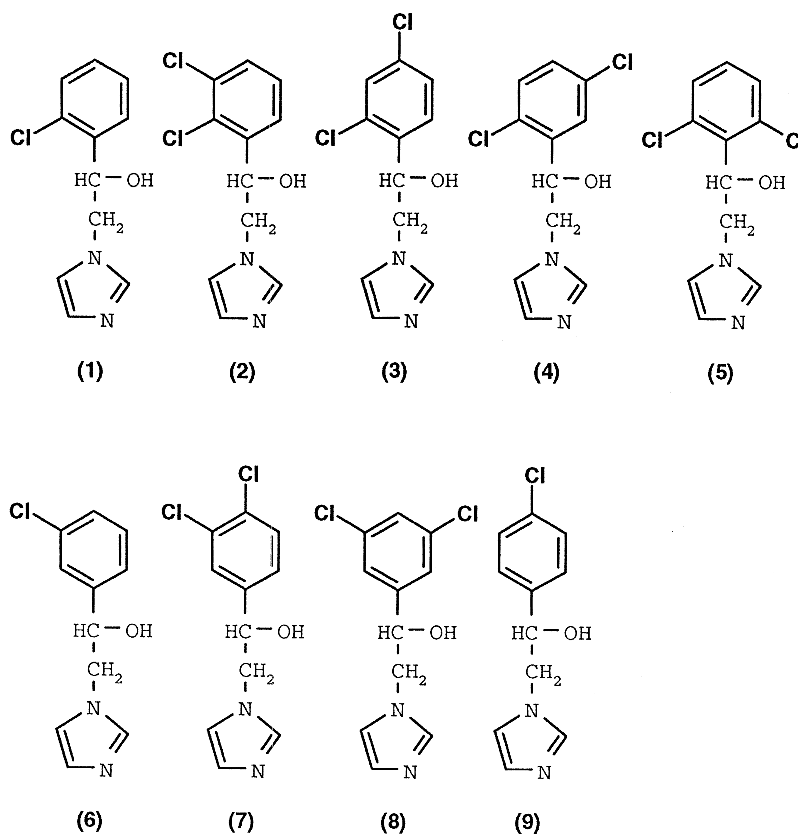

Fig. 1. Tioconazole regioisomer compounds in selectivity test mix.

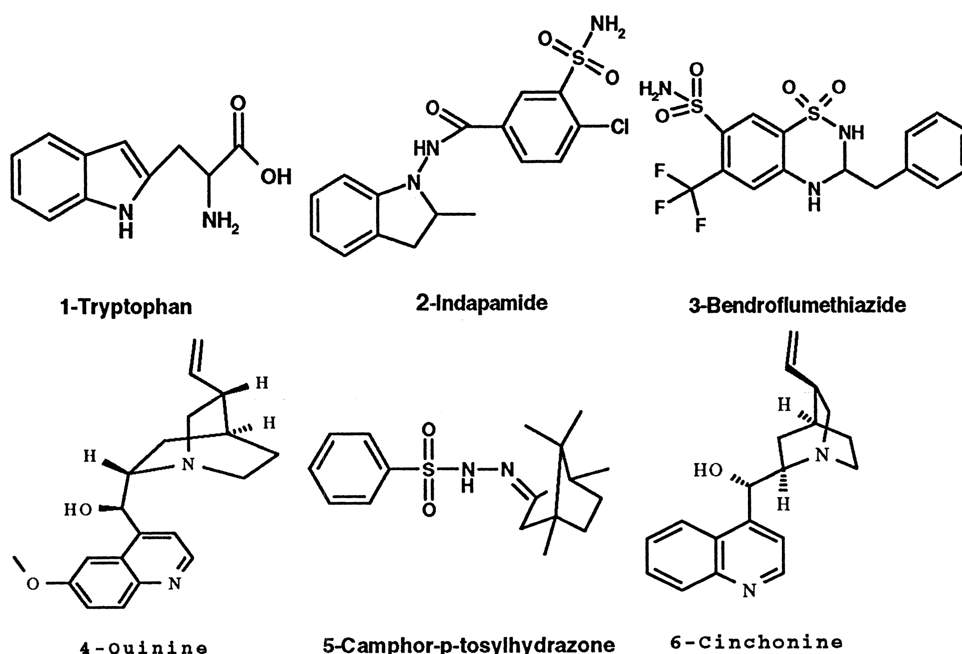

Fig. 2. Sigma samples for analyte hydrophobicity range test.

this generic micellar buffer system against the test parameters of method selectivity and analyte polarity, included a set of tioconazole precursor regioisomers (Fig. 1) and a test mix of six Sigma compounds (Fig. 2).

After testing this method against selectivity for similar compounds and analytes over a diverse range of polarity, this method was made available for routine use by discovery chemists.

3. Results and discussion

3.1. Generic micellar method

The buffer and micelle concentrations required for this generic method need to satisfy the following criteria; selectivity, speed and the option of chiral method development. The SDS and buffer concentrations selected must give stable reproducible current profiles when applying the separation voltage and should allow chiral selectors to be added to the run buffer without resulting in excessive Joule heating [13–15]. As a result, a 20 m*M* borate buffer at pH 9.2 which gives a robust EOF and short run times without generating excessive currents was selected. Also, the addition of 18 m*M* SDS allows selectivity for neutrals without increasing run times excessively. An elution time window of 2.3 min to 9.0 min (mesityl oxide used as EOF marker and sudan III used as micelle marker) enabled 10 min run times to be used. A 30-s flush from 9.5 min was incorporated into the method to allow analytes which migrating outside the elution time window to be detected. These micellar buffer conditions satisfy the critical requirements outlined for a generic separation of acids, bases and neutrals in one short method.

The ease of operation of CE creates the opportunity for preparing OA software provided certain requirements can be met. The criteria specified for a viable OA system fell into three categories; instrument, operator and manager requirements. The key CE instrument requirements were to have an OA compatible instrument and the option to make the software more user-friendly. Key operator requirements were to have a manager access level enabling full instrument control and a user access level which gives restricted access to the customised OA screens.

The design of a simple welcome screen for users with an easy sample logging format and the option to re-integrate data were included as part of the customised OA design specification.

3.2. Open access system

The safe introduction of additional samples to the system whilst it is running is a key aspect of an open access approach. The Hewlett-Packard (HP) ^{3D}CE system does permit samples to be added to the carousel during an electrophoretic separation without cutting out the voltage. The design of this instrument results in the removal of the buffer vials from the carousel during an electrophoretic run. This permits users to place samples directly into the carousel during a separation. The HP ^{3D}CE system was considered to be suitable for OA as it satisfies the key instrument selection criteria.

3.3. Open access software interface

Customisation of HP Chemstation software was carried out to provide an user-friendly system for the synthetic chemist. Following on from the successful systems developed for OA-MS [4,5] and OA-NMR [6], a menu-based sample logging system was devised. A welcome screen prompts the chemist to log-in a sample (see Fig. 3). The chemist then enters the sample name, their laboratory number and selects the CE method required (see Fig. 4). This sequence is repeated for each sample being logged within a chemist's batch. When all the samples for a single chemist's batch (which may be one or as many as forty samples) are logged and placed into the autosampler, the batch information is automatically added to the Sequence Table. Additional sample entries can be made and are appended to the active Sequence Table. Once a sample is logged onto the computer it is registered with a ''waiting'' status and

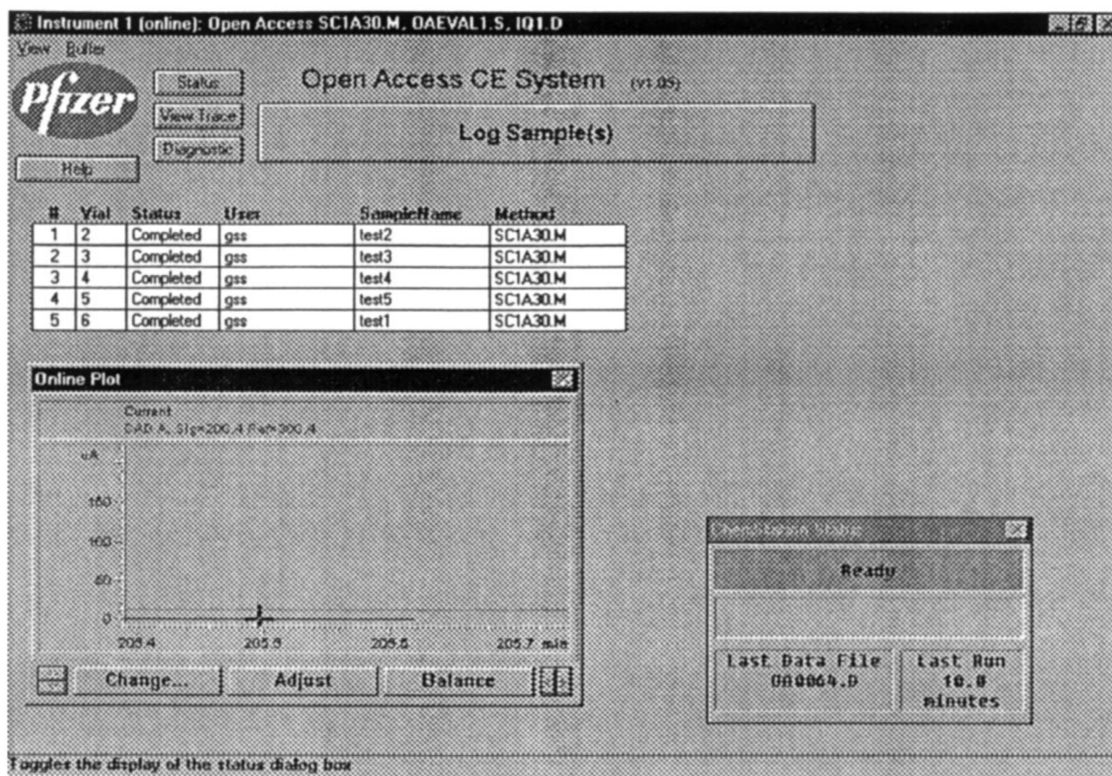

Fig. 3. Open access CE welcome screen.

Fig. 4. Open access CE Sample Input screen.

once added to the active sequence the status changes to ''queued''. During separation the status changes to ''running'' and when the sample has been run the status reads ''completed''. When all the samples in the sequence table have been run the instrument status changes to ''ready'' until another sample is logged on. The system has been designed to enable any instrumental errors to be tracked through accessing the instrument log, which gives date, time, sample and error information. As a result of these

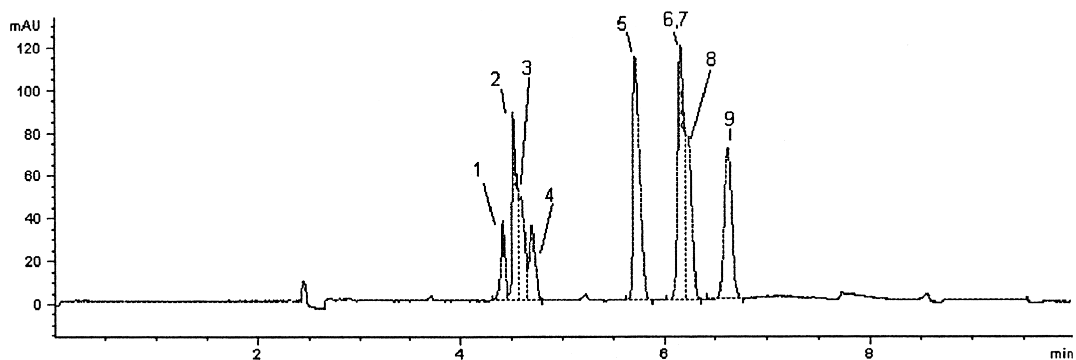

Fig. 5. Tiocanazole test mix separation.

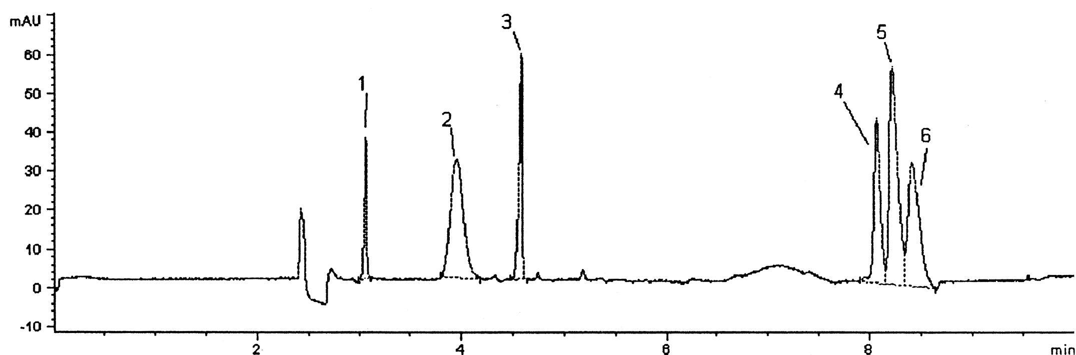

Fig. 6. Sigma test mix separation.

design features the system can run in unattended operation.

3.4. Selectivity test for the generic micellar method

Resolution of eight out of the nine regioisomers of the tioconazole precursor test mix is shown in Fig. 5. Mechanistically this separation results from the differential partitioning of the regioisomers with SDS micelles. One significant factor influencing this partitioning is the inductive effect produced from chlorine substitution around the aromatic ring. This inductive effect imparts differences in hydrophobicity for the mono and dichloro regioisomers which SDS micelles are capable of exploiting. The separation of these closely related structures demonstrates the resolving power of this generic method.

3.5. Analyte hydrophobicity range test for the generic micellar method

The separation of the Sigma test mix is shown in Fig. 6. Baseline resolution is observed for the hydrophilic compounds tryptophan, indapamide and bendroflumethiazide whilst maintaining sufficient selectivity to partially resolve the more hydrophobic analytes quinine, cinchonine and camphor-*p*-tosylhydrazone. A broad indapamide peak is observed in this separation. The peak shape could be improved by increasing the buffer concentration to enhance analyte stacking, however this would result in a longer run time and greater Joule heating within the capillary. This electropherogram illustrates that this micellar method can resolve hydrophilic and hydrophobic analytes in one 10-min run.

3.6. Reaction monitoring by a synthetic chemist using the generic micellar method

A chemist followed the progress of the chemical reduction shown in Fig. 7. Methanolic samples of the reaction mixture run on the OA-CE system gave the electrophoretic profile shown in Fig. 8. UV data for the three separated components enabled tentative

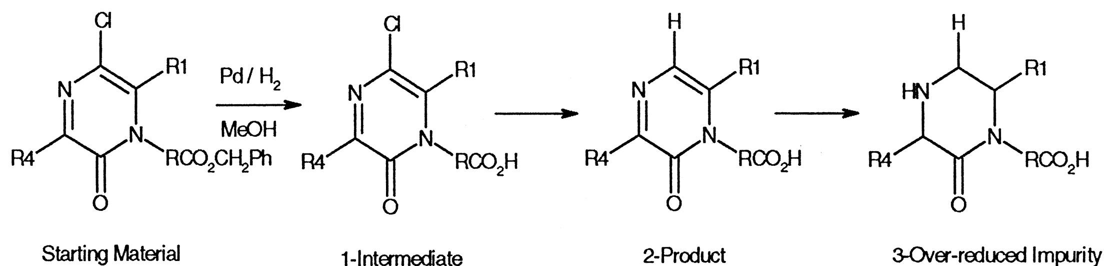

Fig. 7. Proposed hydrogenation reaction scheme.

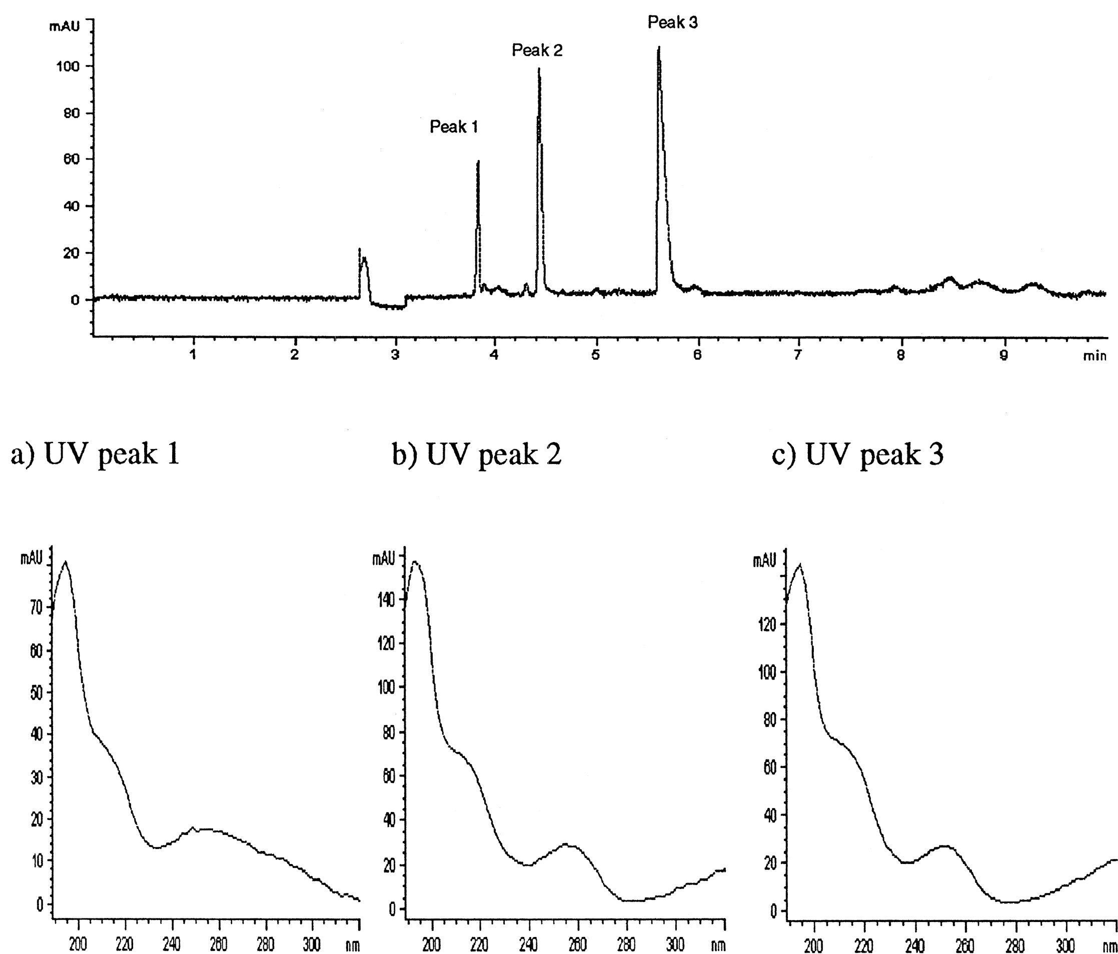

Fig. 8. Hydrogenation reaction mixture separation and UV spectra of Peaks 1–3.

assignment for the peaks observed in the electropherogram.

The UV spectrum of peak 2 is very similar to the starting material, peak 3, therefore it is likely to be the product. The UV spectrum of peak 1 is significantly different to peaks 2 and 3, exhibiting a loss in conjugation in the aromatic ring system. This indicates that the over-reduced impurity is likely to be peak 1. These tentative assignments on the basis of CE and diode array detection data were later confirmed by OA-MS and OA-NMR data. The chemist was able to optimise the conditions for this synthetic step to maximise product yield by carrying out a series of synthetic experiments and using OA-CE as a reaction monitoring tool

4. Conclusions

A system has been devised that allows chemists to walk up and use OA-CE. The micellar buffer used here is not expected to be the optimal separation method, but does provide adequate separations in 10 min. This method was able to resolve eight out of the nine structurally similar tioconazole precursor regioisomers. The Sigma test mix application shows that selectivity is maintained over a broad range of analyte polarity. The potential for OA-CE to solve real problems in Discovery Chemistry at Pfizer has been demonstrated for the reaction monitoring of a chemical hydrogenation. The system described here enhances the breadth of OA techniques in the

chemist's armoury, and significantly reduces the time taken between sample submission and the chemist receiving high quality separation results. The customised OA software developed here can also be used with Chemstation compatible chromatographic systems. The delivery of results via e-mail is currently being evaluated. This OA-CE system provides the opportunity for non-specialists to use CE.

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