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Note

Analysis of tioconazole using high-performance liquid chromatography with a porous graphitic carbon column

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Porous graphitic carbon (PGC) is a strong reversed-phase absorbent with retention characteristics similar to those of alkyl bonded silicas, *e.g.* octadecylsilane (ODS)[1–4]. However, unlike silica gel it offers a number of critically important advantages. These include the absence of residual silanol groups, which can give rise to problems with the elution of amines, insolubility in aggressive aqueous–organic mobile phases at extreme pH values, a homogeneous surface and a reproducible performance from batch to batch. Polymeric packing materials also offer some of these advantages but suffer from other problems such as limited mechanical stability and poorer mass transfer properties. PGC columns would appear, therefore, to offer unique properties which would commend them particularly to the analysis of basic compounds of pharmaceutical interest. The number of literature applications with PGC columns is still, however, very small.

The determination of tioconazole and three very closely structurally related potential impurities is the subject of a monograph in the *United States Pharmacopoeia*[5]. However, the separation is difficult to achieve with ODS reversed-phase columns, requiring long elution times and an aggressive mobile phase (leading to short column lifetimes and requiring that a solvent conditioning pre-column be included). In addition the major hydrolysis product of tioconazole is unretained and cannot be determined. The analysis of tioconazole has been the subject of further investigation and optimisation[6,7] from which it was concluded that a phenyl-bonded silica with an ion-pairing mobile phase provided optimum selectivity within the constraints of a non-aggressive mobile phase. However, it was further shown that to achieve optimised resolution of the low levels expected of the potential impurities it was necessary to modify the detection wavelength from 219 nm, as specified in ref. 5, to 260 nm[7]. This shift to longer wavelength places increased demands upon the detector performance and makes the detection of low levels of the hydrolytic degradation product more difficult.

This paper describes the use of a PGC column to separate tioconazole from the three potential impurities and its main hydrolytic degradation product. It is also shown that the enhanced selectivity offered by the PGC column permits the use of low wavelength detection.

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EXPERIMENTAL

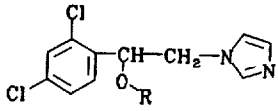
A Model 1090A liquid chromatograph (Hewlett-Packard, Wokingham, U.K.) equipped with a diode-array detector and autosampler was used for the present studies. Detection wavelengths of 220 and 260 nm were used with bandwidths of 10 nm. The column was 10 cm × 0.46 cm I.D. porous graphitic carbon of mean particle diameter 7 μm (ChromatoGraphite; Wolfson Liquid Chromatography Unit, University of Edinburgh, Edinburgh (U.K.). Solvents were HPLC grade (Rathburn Chemicals, Peebles, U.K.) and all other reagents were reagent grade. Tioconazole (I) and its potential impurities (II–IV) and major hydrolysis product (V) were provided by Pfizer Central Research. Solutions were prepared in mobile phase at concentrations of approximately 0.5 mg/ml. Separations were carried out with a flow-rate of 1.5 ml/min and with a column temperature of 40°C.

Comparison chromatograms result from earlier studies with tioconazole; chromatographic conditions are described on the relevant figures.

RESULTS

The difficulties associated with achieving satisfactory resolution of tioconazole (I) from its deschloro (II) and, in particular its 2,5-dichloro (III) and 2-chloro-5-bromo (IV) analogues using the USP method are illustrated in Fig. 1.

Indeed, differentiation in the time domain (differentiating the detector signal with respect to time in order to enhance resolution) has been proposed as an adjunct to this difficult separation[8] to improve resolution and quantitation at low levels. The optimised separation using a phenyl column[7] is shown in Fig. 2. The separation is now faster and the resolution improved but, to detect levels of the impurities III and IV without interference from the tail of the tioconazole peak, it is necessary to use 260 nm



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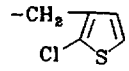

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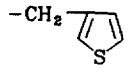

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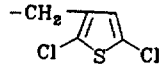

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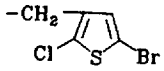

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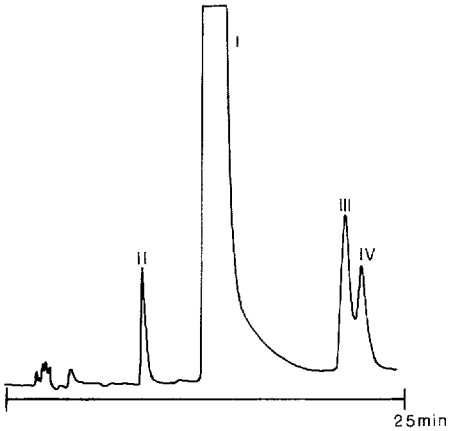

Fig. 1. Separation of tioconazole according to ref. 5. Column (25 cm × 0.46 cm I.D.) of LiChrosorb RP-18 eluted with methanol–acetonitrile–water–ammonia (220:200:190:1, v/v) at 1.5 ml/min and 40°C. Detection at 219 nm.

to capitalise upon spectral discrimination. At 260 nm the longer wavelength absorbance possessed only by compounds III and IV allows their detectability to be maintained while reducing the contribution of tioconazole. Unfortunately, compounds II and V do not possess this longer wavelength absorption and are consequently more difficult to detect. In addition, the ion-pairing mobile phase is more complex and the reproducibility of the separation, with respect to columns from different manufacturers, has not been fully evaluated.

In developing the separation on the PGC column, a simple and robust separation was desired. Retention of tioconazole and its impurities could be obtained with acidic aqueous–organic mobile phases but retention was not accompanied with

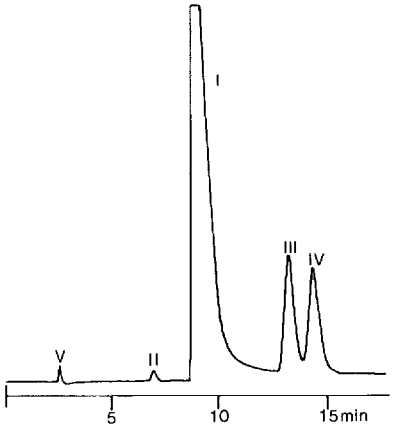

Fig. 2. Optimised separation using 15 cm × 0.46 cm I.D. (5 μm) Hypersil phenyl column[6]. Mibile phase of methanol–acetonitrile–buffer (39:16:45, v/v). Buffer comprises 0.05 *M* triethylamine and 0.025 *M* 1-octanesulphonic acid adjusted to pH 4.0 with orthophosphoric acid. Flow-rate = 1.5 ml/min, column temperature = 40°C, detection at 260 nm. Compounds II–V are approximately 0.5% relative to tioconazole.

resolution. A mobile phase of 35% tetrahydrofuran in water, with 1% ortho-phosphoric acid produced a capacity factor (k') for tioconazole of 5. The relative ease of elution with acidic mobile phases is to be expected with the protonated compounds —tioconazole has a pK_a of 6.4. Greater retention (*i.e.* a higher k' value) and the possibility of improving the selectivity requires the use of mobile phases at pH values greater than 8.4. Unlike conventional silica based columns, PGC columns offer the possibility of working with basic mobile phases and high pH mobile phases were thus used for the remainder of the studies.

All the compounds under investigation were extremely strongly retained with basic mobile phases, neither methanol or acetonitrile being strong enough to elute the tioconazole in less than 20 column volumes of mobile phase. Tetrahydrofuran, however, proved to be a relatively strong modifier[3]. A mobile phase at pH 10.5 of tetrahydrofuran–water (70:30, v/v) containing 1% aqueous ammonia (sp.gr. = 0.880) produced adequate retention which was accompanied by very high selectivity for all compounds (Fig. 3) and permitted a fast separation to be achieved. Such was the selectivity between tioconazole and compounds III and IV that their detection at 0.2% (w/w) relative to tioconazole could be easily accomplished using a detection wavelength of 220 nm. Furthermore, the selectivity obtained with this simple mobile phase precluded the need to undertake a formal programme of mobile phase optimisation. The use of 220 nm also conferred the advantage of enhancing the detectability of compounds II and V, both of which were also easily detected at the 0.2% (w/w) level.

The column efficiencies were not as high as those obtained for silica-based columns, a reduced plate height of 14 being determined for the peak due to III. This lack of efficiency is the subject of further studies but potentially will reduce the ultimate detectability of the late-eluting compounds. However, because of the very high selectivity, both compounds are well resolved from each other and from tioconazole and adequate sensitivity can be maintained. Linearity of determination of all potential impurities was demonstrated over the range 0.2–1.2% (w/w) relative to the tioconazole.

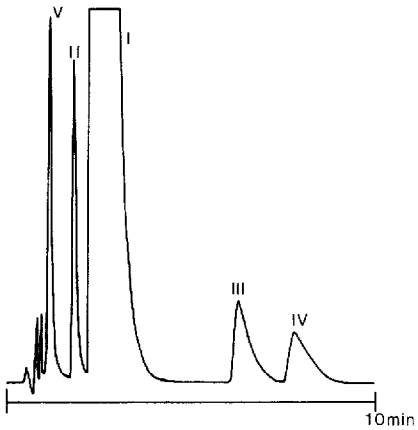

Fig. 3. Separation on 10 cm × 0.46 cm I.D. PGC column. Mobile phase of tetrahydrofuran–water (70:30, v/v) containing 1% ammonia (sp.gr. = 0.880) at 1.5 ml/min with column temperature of 40°C and detection at 220 nm. Compounds II–V are approximately 0.5% relative to tioconazole.

CONCLUSIONS

The use of a PGC column has clearly shown advantages over the more traditional silica-based reversed-phase supports. The hydrolytic stability of the support permits the use of mobile phases at pH values far exceeding those suitable for silica based columns. In the analysis of tioconazole, this stability could be exploited to permit the direct chromatographing of the unionised solutes using a basic mobile phase to obtain high selectivities. No additives were required to mask residual silanol groups and such chromatographic simplicity is likely to find widespread applicability in the separation of basic molecules. The selectivities obtained somewhat surprisingly far exceeded those obtained following extensive separation optimisation on ODS or phenyl-bonded columns. Since PGC columns by their very nature are likely to be highly reproducible, their use in pharmaceutical analysis combines all the advantages of robustness with the possibilities of straightforward method transfer to all those who may need to use it.

ACKNOWLEDGEMENT

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