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Note

Chromatographic analysis of azapropazone and related benzotriazines

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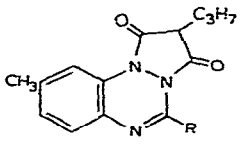
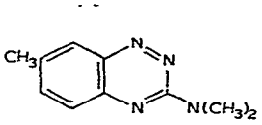
Azapropazone is a new antirheumatic compound which is the active component of Rheumox capsules. The compounds represented by the structural formulae include azapropazone (I) and structurally related compounds. Compound IV is the starting material for azapropazone synthesis and compounds II, III, and V are possible impurities in commercial azapropazone[1]. A gas–liquid chromatomatographic (GLC) method has been described for the analysis of azapropazone in body fluids and evidence has been presented indicating that azapropazone is unstable in solution[2].

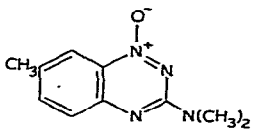
This paper describes chromatographic methods that may be used for the separation and quantitation of the compounds together with data allowing the construction of a quality control method applicable to formulated Rheumox. A study of the stability of certain of these compounds when applied to thin-layer chromatography (TLC) plates is also described.



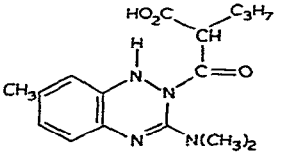
I R = $-N(CH_3)_2$
II R = $-OH$



III



IV



V

EXPERIMENTAL

Materials

Compounds I–V and Rheumox capsules were supplied by Robins (Horsham, Sussex, Great Britain).

The TLC and high-pressure liquid chromatography (HPLC) eluents used are described under Results and discussion.

The solvents used were chloroform, ethyl acetate, cyclohexane, and methanol, all Analar grade, supplied by BDH (Poole, Dorset, Great Britain).

The adsorbent used for TLC plastic sheets was silica gel 60 F_{254}, supplied by Merck (Darmstadt, G.F.R.), that for HPLC Corasil Type II, particle size 37–50 μm, supplied by Waters Assoc. (Milford, Mass., U.S.A.).

Thin-layer densitometry

Samples were dissolved in chloroform and applied to the TLC plates using Microcaps (Drummond). The plates were developed in a Gelman Model 51325-1 equilibrated chromatography chamber. Densitometric determinations were carried out using a flying spot densitometer (Vitatron Model TLD 100) operating under the conditions specified in Table I.

High-pressure liquid chromatography

Samples were dissolved in ethyl acetate and analyses were performed at ambient temperature on a 3 ft. × 0.085 in. I.D. stainless-steel column packed with Corasil II. The eluent flow-rate was 0.6 ml/min generated by a Type 6000 M solvent delivery system (Waters Assoc.). A UV spectrophotometer (Cecil Instruments Type CE 272 with a modified flow cell) monitoring at 255 nm was used as the detector.

RESULTS AND DISCUSSION

TLC on fluorescent-grade silica gel plates using 10% methanol in chloroform as eluent allows the separation of all the potential degradation products of azapropazone although compounds III and IV co-chromatograph (Table I). Compounds III and IV (both brightly coloured) can be easily separated using chloroform alone as eluent, giving R_F values of 0.74 and 0.82, respectively. A complete analysis can be carried out using double development with chloroform as the first eluent followed by 10% methanol in chloroform. As compound IV is not found in commercial azapropazone a single development in methanol–chloroform is suitable for routine analysis of azapropazone and impurities in formulated Rheumox capsules. None of the capsule fill additives interfere with the chromatographic behaviour of the individual compounds. Table I gives the relevant operating conditions of the flying spot densitometer for the analysis of these compounds and it can be seen that the colourless compounds are quantified by measuring their quenching of fluorescence. This means that compounds III and IV are capable of assay at lower levels than the other compounds as the flying spot densitometer is capable of greater sensitivity when operating in the adsorption rather than the reflectance mode. The calibration curves of weight *vs.* peak area were linear for all compounds over the concentration ranges studied and reproducibility from one plate to the next was very good (Fig. 1).

TLC was complicated by the fact that azapropazone is photochemically un-

TABLE I

OPERATING CONDITIONS FOR FLYING SPOT DENSITOMETRIC DETERMINATION OF AZAPROPAZONE AND RELATED COMPOUNDS

Compound	R_F*	*Lamp*	*Mode*	*Filter (nm)*	*Minimum assayable (μg)*
I	0.63	Hg/UVB	Lin(−)	254-L	0.14
II	0.13	Hg/UVB	Lin(−)	254-L	0.38
III	0.97	Tunsten	Log(−)	477	0.043
IV	0.97	Tungsten	Log(−)	477	0.042
V	0.45	Hg/UVB	Lin(−)	254-L	0.23

* Eluent, 10% methanol in chloroform.

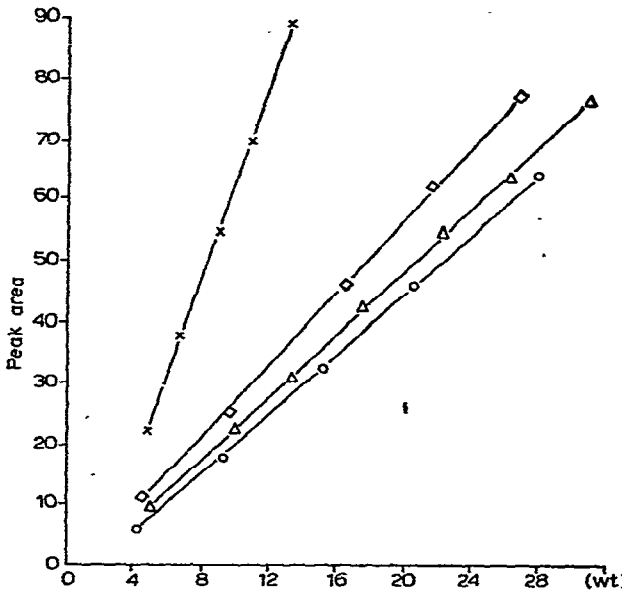


Fig. 1. Calibration curves for the thin-layer densitometry of compounds I–IV. ◇, Compound I ($\mu g \times 10^{-1}$); ○, compound II ($\mu g \times 10^{-1}$); △, compound III ($\mu g \times 10^{-2}$); ×, compound IV ($\mu g \times 10^{-2}$).

stable in solution, an observation previously reported by Leach[2]. This reaction was found to be particularly rapid in bright sunlight and consequently all solutions were freshly made and stored in the dark until use. The chromatograms were developed in subdued light. By TLC the photodecomposition product of azapropazone was shown to be compound III. TLC plates exposed to bright light showed the development of a yellow spot where azapropazone had been applied and this was also shown to be due to the production of compound III, thus indicating a similar photochemical reaction as that found in solution. Further it was found that compound V underwent essentially the same degradation, also affording compound III. Thus in any consideration of the status of compound V as an indicator of the long-term instability of azapropazone in Rheumox some consideration of the relative rates of production of compound III from azapropazone and compound V must be made.

The analysis of the kinetics of photochemical reactions in the solid state is extremely complex but by taking advantage of the observation that the reaction occurs on TLC plates and the fact that the reaction involves the conversion of colourless compounds to a coloured compound a simple method of demonstrating the relative speeds of degradation was devised.

Applications of equal amounts of compounds I and V were made on TLC plates which were then placed in the light beam of a stationary flying spot densitometer, thus using the light source as a means of irradiation and the photodetector with a 477-nm filter as a means of measuring the production of compound III. The recorder thus produces a plot of increase in concentration of compound III against time (Fig. 2). This allows a rough comparison of rates of reaction to be made although

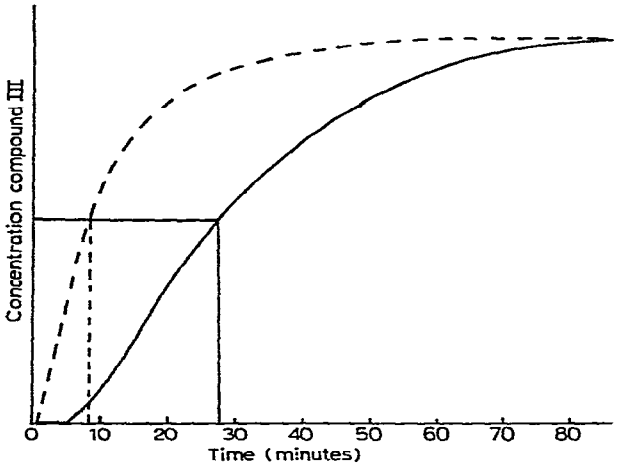


Fig. 2. Rate of production of compound III from azapropazone (– – –) and compound V (———).

its meaning in real terms awaits further investigation. Plots of time against log concentration, reciprocal concentration, and reciprocal of concentration squared indicated that the reaction did not follow simple first-, second-, or third-order kinetics. Additionally, there appears to be a lag period for the degradation of compound V, which is not evident in the azapropazone conversion. The time for 50% conversion was 9.5 min for azapropazone and 28.5 min for compound V.

Incidentally this provides a more sensitive assay for azapropazone as, when

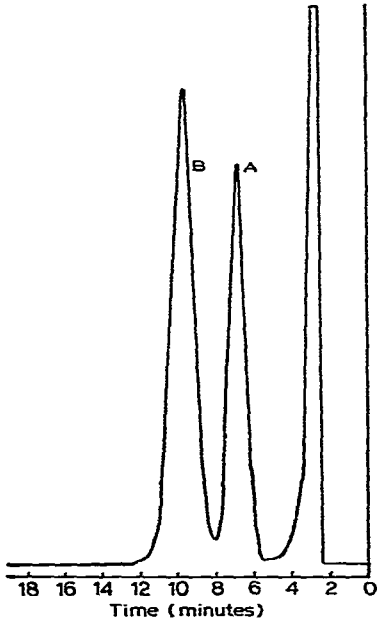


Fig. 3. HPLC analysis of compound III. (A) 0.5 μg of compound III; (B) 1.0 μg of compound IV.

the TLC plate is run and then exposed to bright light, it may be assayed using the transmission mode of the densitometer as for compound III, thus allowing an approximate tenfold increase in sensitivity.

Although commercial azapropazone can be shown by the previously described method to contain less than 0.1% compound III it is important from the viewpoint of long-term stability to look for a more sensitive assay procedure for this compound.

HPLC using Corasil II as adsorbent and 10% ethyl acetate in cyclohexane as eluent allowed the accurate quantitation of compound III in the 0.01 μg range with compound IV providing a convenient internal standard (Fig. 1). Azapropazone itself is not eluted by this solvent system but elution with 10% methanol in ethyl acetate allows the elution of azapropazone with a corrected retention volume of 4.2 ml. The method is capable of detecting 0.1 μg azapropazone and may be used for its assay in body fluids.

Using the HPLC procedure it has been shown that Rheumox capsules stored in amber bottles at ambient temperatures show no significant increase in levels of compound III over a period of one year.

ACKNOWLEDGEMENTS

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2 H. Leach, *Curr. Med. Res. Opini.*, 4 (1976) 35.