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DEVELOPMENT OF A STABILITY-INDICATING ASSAY FOR NAFIMIDONE [1-(2-NAPHTHOYLMETHYL)IMIDAZOLE HYDROCHLORIDE] BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The rational development of a stability-indicating assay for nafimidone [1-(2-naphthoylmethyl)imidazole hydrochloride] is demonstrated. It allows resolution of the decomposition products, obtained both on melting and resulting from decomposition in solution. Ion-pair chromatography was used to elute the solution decomposition products before the parent compound, thus producing maximum sensitivity of detection of these in the presence of undecomposed reactant.

Stability data in terms of first-order rate constants were obtained in two different buffer solutions both from conventional integrated-rate equations using reactant concentration measurements, and by the initial rate method using decomposition product concentrations. In the light of this investigation, additional criteria for stability-indicating assays are suggested, which exploit the sensitivity as well as specificity of high-performance liquid chromatography.

INTRODUCTION

In any study of drug stability, analysis of appropriate species is essential. The stability-indicating assay[1] is defined as an analytical method capable of quantitating unreacted drug uniquely in the presence of its decomposition products. This generally accepted definition, while it ensures specificity, implies that measurement of unreacted drug is the best method for conducting a stability study. This is not necessarily the case. While measurement of unreacted drug allows convenient use of integrated concentration time equations assuming arbitrary orders of reaction, suggestions have been made in the literature that quantitative measurement of decomposition product may afford a better method for evaluating the stability of a drug[2–4]. Such suggestions have not previously been supported by experimental work to any extent. By far the majority of assays in the literature, claimed to be stability indicating, are designed to quantitate undecomposed drug[5–8]. Recently, however, a few reports have appeared where drug stability has been assessed in terms of rate of appearance of decomposition products[9,10]. Estimation of decomposition products rather than undecomposed drug can offer several potential advantages over the analysis of intact drug. However, it also requires more stringent requirements for an assay to be classed as

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stability indicating. The potential advantages may be summarised as follows:

(1) Using the initial rate method, rate constants for a decomposition reaction producting a single decomposition product can be obtained with precision comparable to conventional integrated methods in a much shorter time[11].

(2) The extent of reaction which must be studied is within the realistic shelf-life extent of decomposition, generally accepted as 10%[12].

(3) Rate constants are obtained unique to particular decomposition pathways rather than composite values.

(4) In cases where a reaction leads to a toxic or potentially toxic decomposition product, direct measurement of this product allows the formulation of a suitable limit test independent of the shelf life of the drug[13].

(5) A study of the rate of appearance of different product species can provide information on routes of decomposition occurring under different conditions.

The assay method applied must, in addition to being specific for the undecomposed drug, conform to the following criteria.

(1) Be capable of resolving and detecting all decomposition products leading to quantitation if standard compounds are available.

(2) Be designed to elute reaction products before undecomposed drug for maximum sensitivity of product measurement.

(3) Be capable of such resolution and quantitation for different products formed during different decomposition reactions of a drug.

It is the purpose of the present work to describe the development of such a stability-indicating assay for nafimidone, 1-(2-naphthoylmethyl)imidazole hydrochloride, a compound for potential anti-epileptic use, and to discuss how this assay conforms to the suggested criteria. It is demonstrated how elution order can be manipulated using current ideas of ion pairing[14]. The stability of this compound in the solid is also reported as is its stability in solution at several temperatures and values of pH.

EXPERIMENTAL

Chromatography was carried out using a Waters Assoc. (Northwich, U.K.) pump and M441 detector. Columns were 100 × 2 mm, slurry packed with ODS Hypersil, and injection was by a Rheodyne (Cotati, CA, U.S.A.) 7125 valve and 20 μl loop. UV spectra were obtained on a Cecil (Cambridge, U.K.) 588 recording spectrophotometer, and molecular weight determination in solution was obtained using a Mechrolab (Mountain View, CA, U.S.A.) vapour pressure osmometer Model 301.

Nafimidone (ND) was kindly donated by Syntex Research (Edinburgh, U.K.). Sodium laurylsulphate (SLS) and octanesulphonic acid (OSA) were obtained from Fisons (Loughborough, U.K.), tetraethyl ammonium (TEA) and tetrabutyl ammonium (TBA) bromides from Aldrich (Gillingham, U.K.) and cetrimide (CTAB) from Thornton & Ross (Huddersfield, U.K.). Acetonitrile was obtained from Rathburn Chemicals (Walkerburn, U.K.) and water was purified using a Millipore (Harrow, U.K.) Milli-Q System. All other chemicals were of AnalaR or equivalent grade.

RESULTS AND DISCUSSION

Preliminary measurements

Information from the suppliers indicated ND (Fig. 1) to be a hydrochloride salt with a pK_a value of 6.48. To simulate decomposition in the solid the compound was heated just to melting in order to produce thermal decomposition. This resulted in the production of a single thermal product, designated TP. Purification of TP was carried out by recrystallisation from water. The UV spectrum for TP was almost identical with that of the parent compound in terms of λ_{max} and absorptivity, but the molecular weight in solution, determined by vapour pressure lowering was approximately twice that of ND, indicating possible dimerisation. However, mass spectra of this product did not show a m/z peak at or near the expected dimer value.

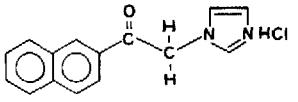

Fig. 1. Structure of nafimidone.

Storage of ND in buffer solutions at elevated temperatures, followed by chromatography of the resulting solution, showed that the compound was relatively stable. However, at high temperatures after long periods of time, three decomposition products could be detected. These compounds could not be recovered in sufficient quantity for complete characterisation. One of these has been tentatively identified from its UV spectrum and chromatographic properties as 2-napthoic acid.

Chromatography

It has been shown previously that the variation of retention with ion-pairing agents depends upon the ionic character of the solute at particular pH[15] and that differences in ionic nature can be exploited to produce differences in selectivity[14]. To examine the ionic character of the decomposition products the variation of their retention, expressed as the column capacity factor, was examined at pH 2 in SLS as pairing ion and at pH 7 using TBA bromide. The results are shown in Figs. 2 and 3. Fig. 2 shows the characteristic maximum expected for ND as a fully protonated base. The thermal decomposition product also shows such a maximum, indicating that this is also a basic species. The three products, P_1, P_2, and P_3, observed following decomposition in aqueous solution show a consistent decrease with increasing SLS concentration, which is characteristic of neutral or fully protonated acidic species with this pairing ion. Fig. 3 confirms these results. ND and TP show decreasing retention with increasing TBA concentration, while the products, P_1, P_2, and P_3, behave as acid anions at this pH, showing the characteristic maxima.

As has been discussed in a previous paper[14], while the shapes of such capacity factor pairing-ion concentration curves are characteristic of the ionic nature of the solutes involved, the magnitudes of the capacity factors depend upon the inherent hydrophobicity of the solutes and on the concentration and hydrophobicity of the pairing ion used. Fig. 2 also indicates that the product of thermal decomposition is much more lipophilic than ND, and its greater retention produces less sensitive de-

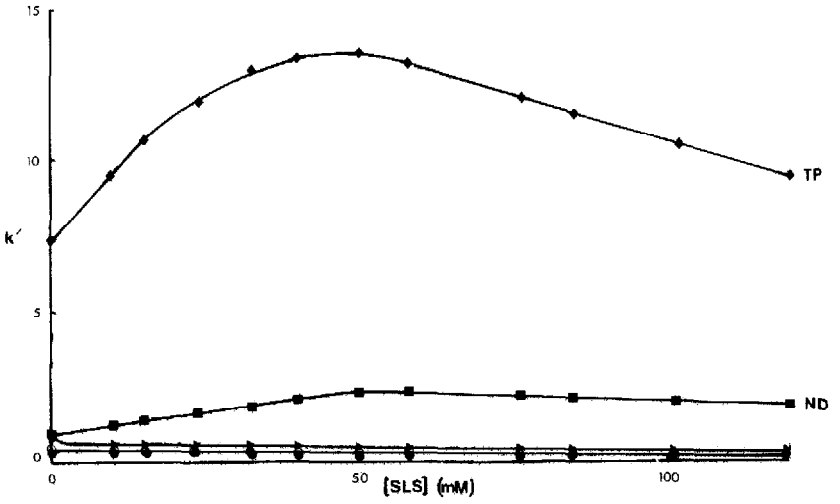

Fig. 2. Variation of capacity factor for nafimidone, ND, its thermal decomposition product, TP, and its three acidic decomposition products, P_1 (▶), P_2 (●), and P_3 (○), as a function of sodium laurylsulphate, SLS, concentration. Solvent acetonitrile–0.02 *M* phosphate buffer (pH 2) (50:50). Column, 100 × 5 mm, 5 μm ODS Hypersil; flow-rate, 2 ml min^{-1}.

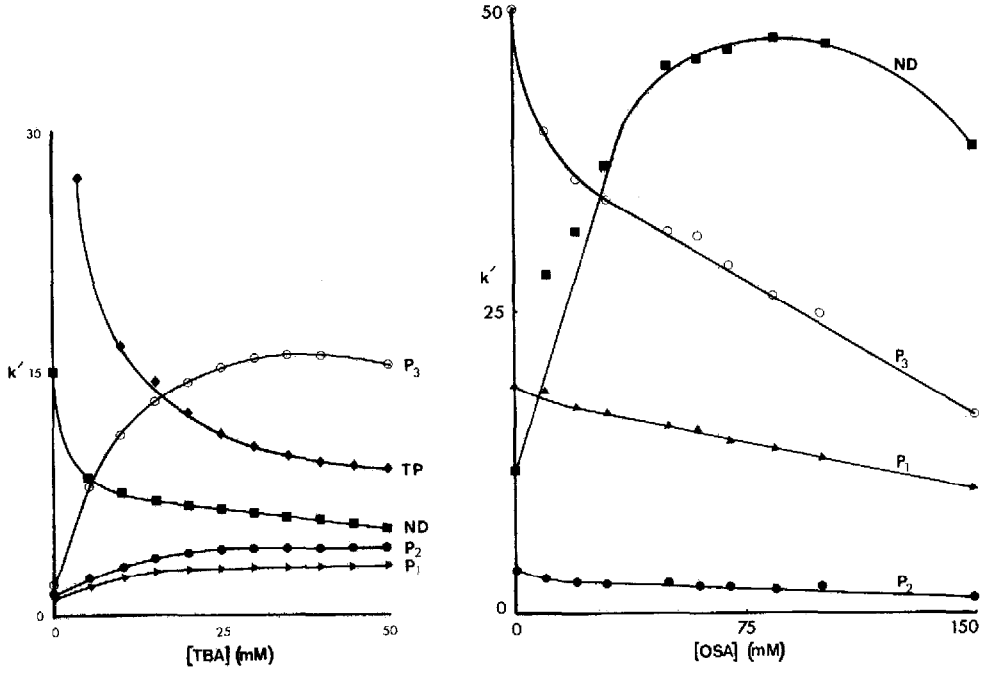

Fig. 3. Variation of column capacity factor for ND and decomposition products as a function of TBA concentration. Solvent acetonitrile–0.04 *M* phosphate buffer (pH 7) (25:75). Column, 100 × 2 mm, 5 μm ODS Hypersil; flow-rate, 0.5 ml min^{-1}.

Fig. 4. Variation of column capacity factor for ND and acid decomposition products as a function of OSA concentration. Solvent and column as in Fig. 3.

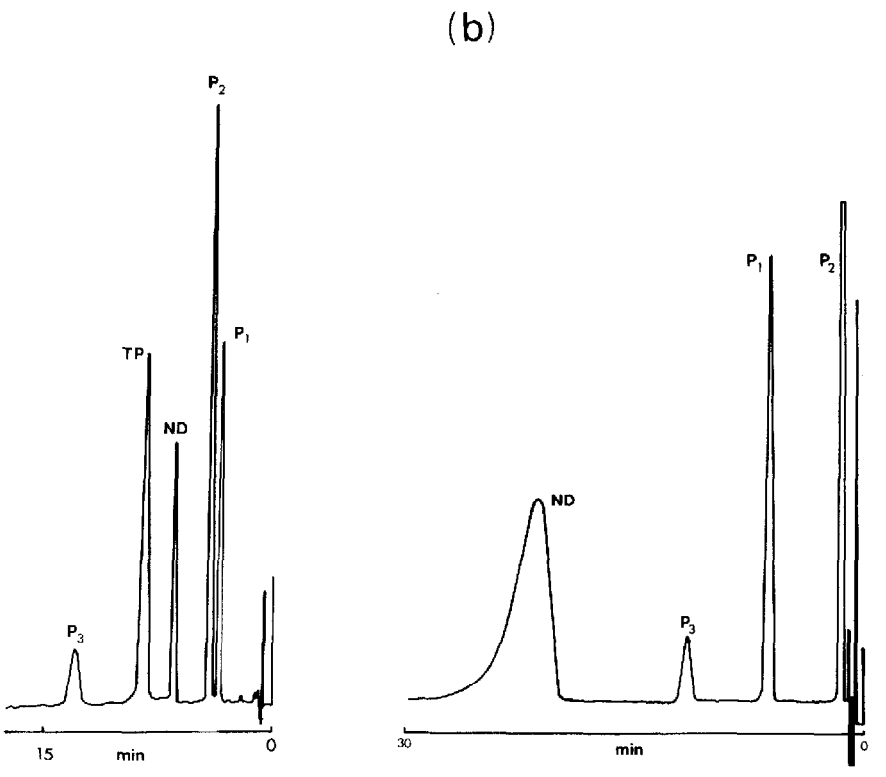

Fig. 5. Specimen chromatograms of ND and its decomposition products (a) using TBA pairing ion at 2 m*M* (b) using OSA at 85 mM.

tection. In addition, the resolution among the acidic products was found to be inadequate for reliable quantitation using any concentration of SLS. Use of OSA at a lower organic modifier concentration resulted in the curves shown in Fig. 4. In this system, the acidic decomposition products were retained sufficiently to be completely resolved and eluted before the parent drug. However, the thermal decomposition product was highly retained. In subsequent measurements of the decomposition of this drug in solution, no thermal product could be detected by any chromatographic system, so that the ion pairing–organic modifier system, shown in Fig. 4, forms the basis of an adequate stability-indicating assay. For initial examination of the decomposition reaction the TBA system demonstrated in Fig. 3 was used. This, while capable of resolving all species detected, had the disadvantage of eluting P_3 after the parent drug. Other cationic pairing ions investigated, CTAB and TEA, showed no improvement over this system. Specimen chromatograms of the TBA and OSA systems are shown in Fig. 5. Similarities between the UV spectra of P_1 and 2-napthoic acid indicate the possible identity of compound P_1. Using the chromatographic systems described above, identical variations of capacity factor with pairing-ion concentration were found for both P_1 and 2-napthoic acid. This was taken as adequate identification of this decomposition product[16]. The relevant curves are shown in Fig. 6.

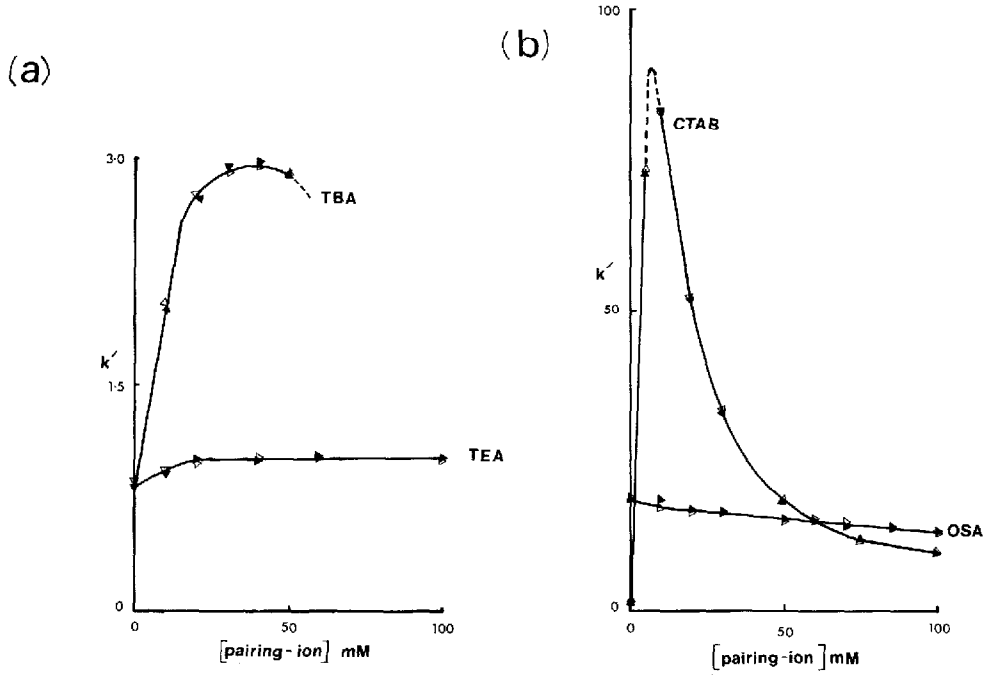

Fig. 6. Variation of column capacity factor with pairing-ion concentration for decomposition product P_1 (▶) and 2-naphthoic acid (▷) (a) using pairing ions TBA and TEA (b) using CTAB and OSA.

Quantification

Using the TBA solvent system the concentration (Conc.)/peak-height (Pk.Ht.) relationships for ND, TP, and P_1 were determined as:

$$\text{ND Pk.Ht.(mm)} = 6.34 \cdot 10^5 \text{ Conc. } (M) - 6.63$$
$$\text{TP Pk.Ht.(mm)} = 3.57 \cdot 10^4 \text{ Conc. (\%, w/v)} - 36.45$$
$$P_1 \text{ Pk.Ht.(mm)} = 1.27 \cdot 10^6 \text{ Conc. } (M) + 3.15$$

The ND sample supplied showed evidence of the presence of P_1, P_2, and P_3 at 0.005 a.u.f.s. This level of P_1 (2-napthoic acid) in the parent drug was determined as 0.08%w/w. While no thermal decomposition product could be detected in the material supplied, the assay method was adequately sensitive for the quantitation of this reaction product at less than 1% decomposition. The relative standard deviations for measurement of the various peak heights were determined as ND (1.6%), P_1 (2.0%), P_2 (1.5%), and P_3 (1.1%).

Stability measurements

Solid state. Prolonged storage of ND at 130°C for several weeks showed no evidence of production of any of the decomposition products when the samples were analysed using any of the solvent systems used. No detectable decrease in the concentration of the parent compound was observed, and it was concluded that the dimerisation reaction suggested by the preliminary results occurred only on melting.

Solution. The rate of decomposition of ND was determined in solutions buffered at various pH values by two different buffer systems.

(a) In phosphate buffers the reaction proceeded at a rate fast enough at 40–90°C to enable the reaction to be followed by analysis of the unreacted ND with adequate precision. In this buffer a continuous increase of the three acidic products was observable.

(b) In McIlvaine buffers, which include citrate to provide constant ionic strength, the decomposition of ND was much slower, and only the major peak, designated P_2, could be seen increasing, although all decomposition products could be detected as impurities at the beginning of the decomposition. Even at 90°C, the decrease in ND concentration was insufficient to obtain adequately precise rate constants by using ND concentration. In this case, the assumption was made that the only decomposition reaction occurring was that of ND to P_2. The increase in peak height of P_2 was measured after a reaction time of 100 h at 90°C when the concentration of ND had decreased appreciably *ca.* 17% as a result of decomposition.

This allowed estimation of the proportionality constant, K, in the equation

$$\Delta \text{ Peak height of } P_2 = -K\Delta\,[\text{ND}]$$
$$= K\Delta[P_2]$$

The molar concentrations of P_2 could thus be determined in the reaction mixture at extents of reaction where a ND decrease would not be detectable.

Table I shows the values of the rate constants for the decomposition of ND in phosphate buffer at various temperatures and pH values, obtained by measuring the rate of ND concentration decrease. Table II lists the rate constants for the decomposition of ND to P_2 in McIlvaine buffer, these values being obtained by measuring the rate of P_2 concentration increase with time at low extents of reaction. These values are quoted as conventional first-order decomposition rate constants,

TABLE I

FIRST-ORDER RATE CONSTANTS FOR THE DECOMPOSITION OF NAFIMIDONE AT DIFFERENT TEMPERATURES AND pH VALUES

Temperature (°C)	*pH*	*Rate constant ($k \times 10^2$) for ND decomposition (h^{-1})*
90	4.75	1.04
90	5.60	1.05
90	6.35	1.15
90	6.59	1.13
90	7.00	2.55
90	7.10	3.19
90	7.40	4.16
90	7.75	4.94
80	7.10	1.50
65	7.10	0.19
40	7.10	0.009

TABLE II

FIRST-ORDER RATE CONSTANTS FOR THE DECOMPOSITION OF ND TO P_2 OBTAINED BY THE INITIAL-RATE METHOD

Temperature (°C)	*pH*	*Rate constant ($k \times 10^4$) for decomp. of ND to P_2 (h^{-1})*
90	2.00	1.86
90	2.70	4.53
90	3.95	0.86
90	4.35	0.57
90	5.35	2.02
90	5.65	2.11
90	5.90	4.98
90	6.50	8.07
90	6.73	10.30
90	7.59	18.00
90	8.10	27.20
80	5.90	2.39
70	5.90	1.05
60	5.90	0.44

obtained by applying the relationship

$$k_0 = k_1 [N]_0$$

where k_0 is the observed linear or zero-order increase of P_2 concentration with time, k_1 the first-order rate constant, and $[N]_0$ the initial molar concentration of ND. This latter procedure cannot be applied to the phosphate buffer decomposition. The individual peak heights cannot be related to concentration values in the absence of identification of the decomposition products and without the availability of standard samples when more than one product is formed.

CONCLUSIONS

The present work shows that it is relatively easy to determine ND by reversed-phase HPLC, a method which is specific for ND in the presence of its decomposition products. This need not involve the use of ion-pairing agents. However, modification of elution characteristics, by use of current ideas on ion pairing can provide solvent systems that are capable of separating individual decomposition products both from one another and from the parent drug. It has also been possible in this instance to arrange the elution order so that the decomposition products detectable in solution are eluted before the parent peak. By using increased sensitivity of detection of the major product it has been possible to obtain rate constants for the decomposition at extents of reaction where the inherent imprecision of reactant concentration has rendered the conventional application of integrated rate equations impractable.

In the case of this particular investigation, because not all of the reaction products have been identified, the initial rate method of determining rate constants

has only been applied to a limited extent. However, chromatographic monitoring of the reaction using decomposition product peaks has demonstrated the complexity of the reaction in solution and shown the stability of the drug in the solid state more reliably than measurements of ND.

The limited stability data reported here serve to indicate the potential advantages of examining reaction products quantitatively as an intrinsic part of a drug-stability investigation. These advantages require that for a drug assay to be considered truly stability indicating, it should be capable of measuring product as well as reactant concentration changes due to decomposition, *i.e.* the sensitivity as well as the specificity of HPLC should be exploited.

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