High-performance liquid chromatography procedure for the determination of purity of di-N-n-propylamine

Sean D. McCrossen, Robert G. Giles, Paul W. Oxley and James V. McArdle

*Analytical Sciences, SmithKline Beecham Pharmaceuticals, Tonbridge, Kent TN11 9AN (UK)*

ABSTRACT

Di-N-n-propylamine (DNP) is used in the final step of the synthesis of ropinirole (SK&F 101468-A). Trace impurities in DNP give rise to significant impurities in the raw drug substances. A high-performance liquid chromatographic (HPLC) method for the trace analysis of the impurities in DNP was developed and validated. The trace enrichment method uses a simple derivatisation reaction with oxindole analogous to the final step of the ropinirole synthesis. DNP samples of varying purity were reacted with oxindole and were used in the preparation of ropinirole. The products were then assayed by the HPLC method. A specific impurity in ropinirole was shown to correlate well with the product of the reaction with oxindole. From the correlation data a calibration curve was constructed which enabled a specification for DNP to be determined based on the reaction with oxindole. Thus, the HPLC method could be used to assess the acceptability of DNP for use in the ropinirole synthesis.

INTRODUCTION

Ropinirole, 4-[2-(dipropylamino)ethyl]-1,3,dihydro-(2H)-indol-2-one monohydrochloride, SK&F 101468-A **I**, is a dopamine agonist used in the treatment of Parkinson's disease. Di-N-n-propylamine (DNP) is used in 10-fold excess in the last step of the synthesis of ropinirole [1] as shown in the reaction below (1).

(1)
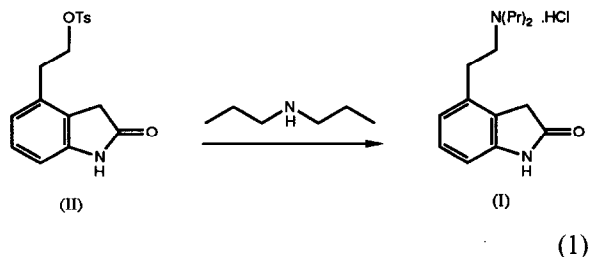
We developed a sensitive gas chromatography–mass spectrometry (GC–MS) procedure to determine the impurity profile of DNP (Fig. 1). However, trace amounts of unknown impurities in the DNP can lead to detectable levels of impurities in the final product. The trace impurities in the reagent become significant because of the 10-fold excess used. Different reaction kinetics may also contribute to the importance of trace impurities. References concerning the determination of impurities in or purity of DNP are not apparent in the literature. Using GC and GC–MS techniques, we have not yet been able to correlate any single impurity in DNP with impurities that appear in **I**. Further, we have shown by GC–MS that propionaldehyde, a suspected impurity, was absent from a batch of DNP used in the manufacture of impure **I**. We would normally perform a "use-test" on a reagent to judge its acceptability. A use-test is a small scale reaction that mimics the reaction performed in the manufacturing plant. If the use-test gives product that meets specification then the reagents may be considered acceptable. Use-tests on DNP are impractical for

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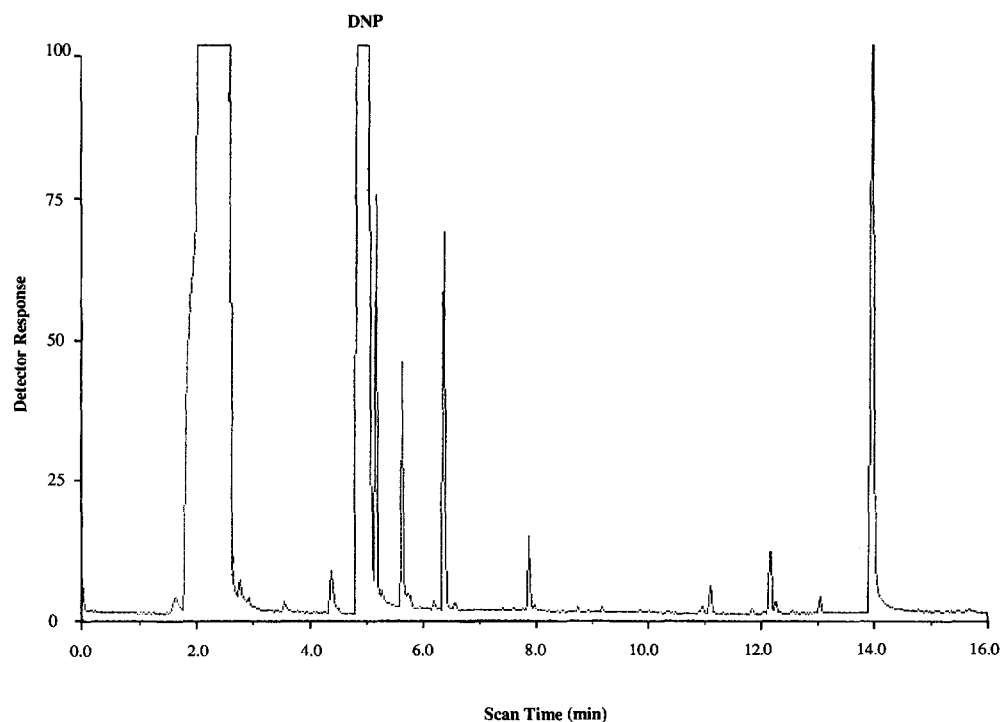*Correspondence to:* S. D. McCrossen, Analytical Sciences, SmithKline Beecham Pharmaceuticals, Tonbridge, Kent TN11 9AN, UK.

Fig. 1. Gas chromatograhic separation of impurities in DNP using electron impact (EI)-MS detection. Column: DB-5, 5% phenyl methylsilicone 30 m × 0.25 mm. Program: 30°C hold 1 min then ramp to 250°C at 10°C/min. Injection: 1 μl splitless at 200°C. Flow-rate: helium at 1.0 ml/min. Solvent: dichloromethane.

the following reasons: (1) A number of side reactions with the tosylate precursor **II** are possible. (2) Testing a large number of batches by this method is time consuming. (3) The use-test involves a two-phase reaction under reflux at 80–85°C and maintenance of an oxygen-free atmosphere throughout the reaction. DNP is sensitive to oxygen and degrades upon exposure to air. Oxindole **III** reacts with the unknown impurities in DNP to give the derivatisation products **IV** and **V**.
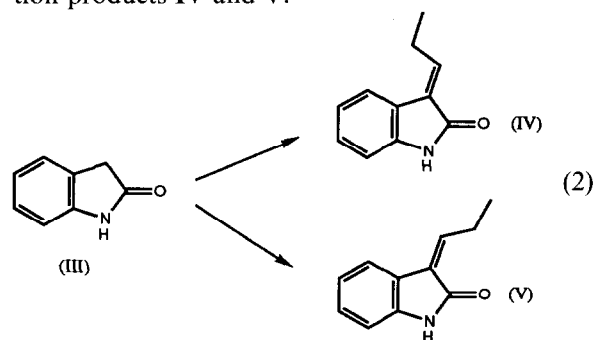
The major product is **IV**, (*E*)-3-propylidene-1,3-dihydro-(2H)-indol-2-one. The amount of **IV** formed from the reaction with oxindole is determined by high-performance liquid chromatography (HPLC) using an external standard. One of the impurities that arises in the final step of the ropinirole synthesis is 4-[2-(dipropylamino)ethyl]-3-propylidene-1,3-dihydro-(2H)-indol-2-one monohydrochloride, **VI**. A single batch of DNP may be used in reaction **1** and in reaction **2**. In this report we show that the amount of **VI** produced in reaction **1** correlates well with the amount of **IV** produced in reaction **2**. Conditions for reaction **2** were optimised and the HPLC

(2)
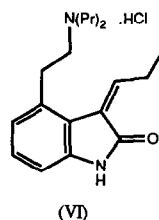
method used to quantitate **IV** was validated. Thus, the model reaction of DNP with oxindole may be used to assess the quality of DNP for use in the synthesis of ropinirole.

EXPERIMENTAL

Apparatus

A Grant Model W6 thermostatic bath circulator (Cambridge, UK) was used to thermostat the reactions and samples. HPLC separations were performed on a Beckman System Gold HPLC apparatus (High Wycombe, UK) with either a Model 166 variable wavelength detector or a Model 168 diode array detector, a Model 126 pump and either a Model 506 or 507 autosampler. A Jones Chromatography Model 7961 column oven (Hengoed, UK) was used to thermostat the column. Data collection and reduction were performed with the Perkin-Elmer Class 2000 system (Beaconsfield, UK). The chromatographic separations were performed on a Kromasil C_8 25 cm × 4.6 mm I.D. stainless steel column with 5-μm packing (Technicol, Stockport, UK).

Reagents

HPLC-grade solvents (Romil Chemicals, Loughborough, UK) were used throughout the investigations. Purified water was obtained from a Milli-Q water purification system (Millipore, Watford, UK). DNP was obtained from BASF (Cheadle, UK). Oxindole was obtained from Merck (Lutterworth, UK). Phosphoric acid (AR) was obtained from Aldrich Chemicals (Gillingham, Dorset, UK). Ammonium acetate (HPLC grade) was obtained from Fisons (Loughborough, UK). Standard compound **IV** and **V** were prepared using the method of Tacconi [2] by reacting oxindole with propionaldehyde. The E and Z isomers were separated and purified by flash column chromatography. The stereochemical structures were established using ^{13}C NMR spectroscopy. The coupling constant for $^3J_{CH}$ was found to be 12.8 ± 1 Hz for the Z and 5.5 ± 1 Hz for the E isomer. Standard compound **VI** was prepared and purified using an in-house method based on the reaction of oxindole with propionaldehyde [3]. The stereochemical structure was established using single crystal X-ray crystallography (SmithKline Beecham Pharmaceuticals, Upper Merion, PA, USA).
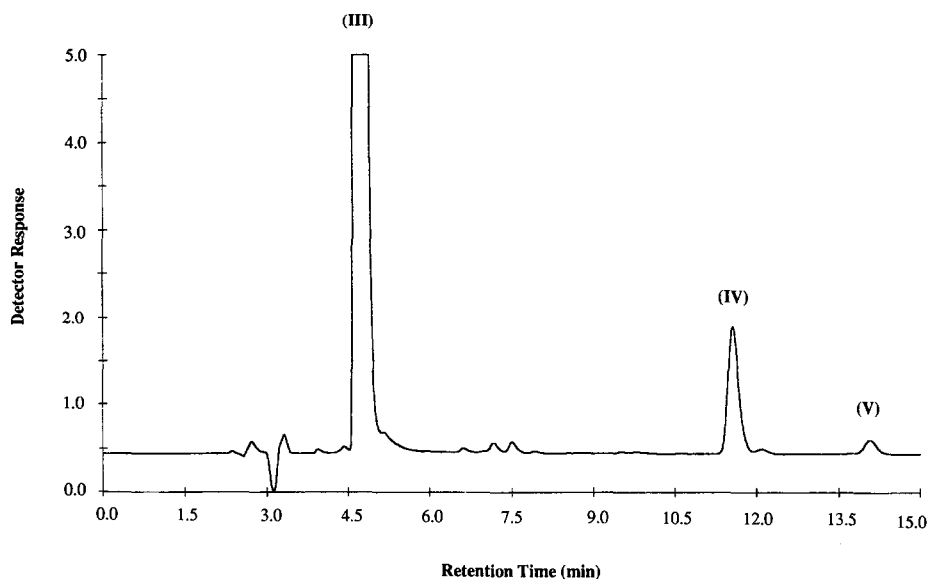

Fig. 2. Chromatograhic separation of **IV** in the oxindole reaction mixture with degraded DNP. Column: 5-μm Kromasil C_8 (25 cm × 4.6 mm I.D.). Mobile phase: 0.05 M ammonium acetate (pH 2.5)–[methanol–acetonitrile (30:70)], (55:45). Detection: 250 nm. Flow-rate: 1.0 ml/min.

Methods

Reaction of DNP with oxindole. Oxindole (0.5 g) was dissolved in 50% aqueous methanol (30 ml) to give an oxindole stock solution. DNP (200 μl) was reacted with oxindole stock solution (200 μl) in 50% aqueous methanol (total volume 25 ml). Reaction time and reaction temperature were varied to obtain a rapid reaction and good stability of the product.

Chromatographic determination of IV. Solvent A was prepared by dissolving ammonium acetate (3.85 g) in water (1000 ml) and adjusting the pH to 2.5 with phosphoric acid. Solvent B was prepared by mixing acetonitrile (700 ml) with methanol (300 ml). The solvents were filtered and degassed before use. An isocratic program (45% solvent B) was performed for 15 min using a 1.0 ml/min flow-rate and UV detection at 250 nm. Oxindole reaction solution (10 μl) was injected on to the pre-equilibrated column at 40°C in duplicate. A typical chromatogram is shown in Fig. 2. A standard solution of IV was prepared by dissolving 500 μg in 100 ml of 50% aqueous methanol and chromatographed.

The weight in μg of IV formed per g of DNP was determined using the equation:

$$\mu g \text{ of } \mathbf{IV} \text{ per g DNP} = \frac{APV_s}{RVD}$$

where:

A = average area under the peak of compound IV in the sample chromatogram

P = purity of reference standard of compound IV expressed as a decimal fraction
= 0.982 for the current standard

V_s = volume of reaction solution (25 ml)

R = average response factor of compound IV from the chromatograms of standard solution [area/concentration (μg/ml)]

D = density of DNP (0.74 g/ml)

V = volume of DNP used in the reaction (0.2 ml)

Preparation of DNP of varying purity. DNP was rendered impure by refluxing in air for 18 h. DNP was purified by refluxing with oxindole in the absence of air then distilling. DNP mixtures of pure and impure samples were prepared volumetrically to give reagent with varying purity. The samples were mixed in volume ratios pure:impure of 100:0, 75:25, 50:50, 25:75 and 0:100.

Reaction of DNP with II. DNP of varying purity (10.5 ml, prepared as described above) was mixed with II (2.5 g) and water (18 ml). The mixture was thoroughly degassed with nitrogen, then heated at reflux for two hours. The mixture was then acidified with concentrated HCl to pH 1 and cooled under nitrogen to give ropinirole in solution. The whole was then diluted quantitatively to 100 ml with acetonitrile–water (70:30). A solution for analysis was prepared by diluting a 5-ml aliquot to 100 ml with 16% solvent B in water.

Chromatographic determination of VI in I. The same column and conditions as for the chromatographic determination of IV were used. An isocratic elution by 16% solvent B for 16 min was followed by a linear gradient to 80% solvent B over 20 min. Ten μl of the solution for analysis prepared as described above were injected in duplicate. The chromatogram obtained from one solution is shown in Fig. 3. The concentration of VI is expressed as a peak area ratio relative to the peak area for I. This method has been fully validated in terms of linearity, precision, peak homogeneity, and ruggedness. The limit of detection of VI in I is 0.01 area %.

RESULTS

Kinetics of reaction between DNP and oxindole

The course of the reaction was followed by sampling a reaction mixture at regular intervals and determining the content of IV by HPLC.

The reaction proceeds at room temperature but is unacceptably slow. The effect of varying the temperature on the kinetic profile is shown in Fig. 4. At 30°C the reaction was not complete until about 8 h. At 40°C significant decomposition of the product was detected by a reduction in peak area at 6 h. At 35°C the reaction is complete after 5 h with some decomposition detected after about 6 h. Thus reaction for 5 h at 35°C was chosen as optimal.

Reaction mixtures were chromatographed within 30 min of completion of reaction. The stability of the reference standard IV was tested in aqueous methanol at room temperature by monitoring the UV absorbance at 250 nm over a 16-h period. The results showed that IV in 50% aqueous methanol was unstable and that standard solutions need to be used within 6 h of preparation.
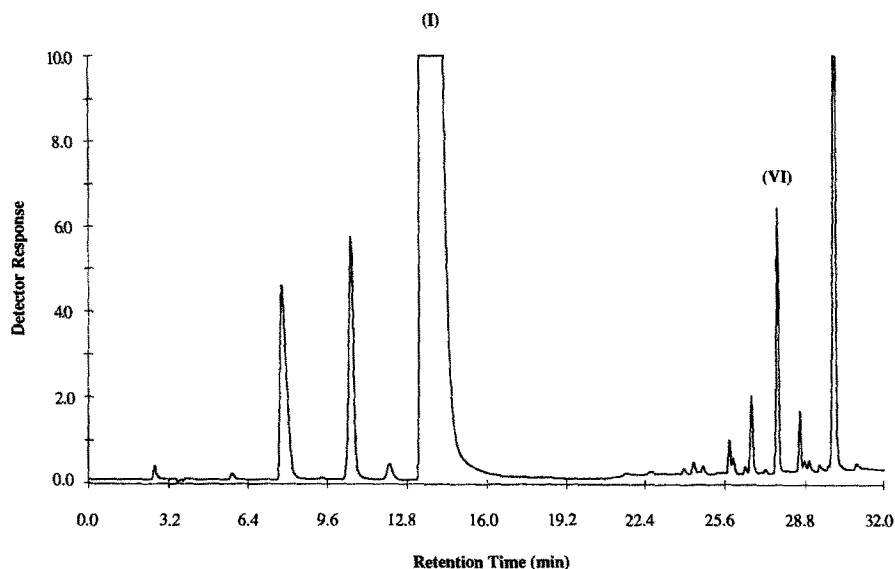
Fig. 3. Chromatograhic separation of impurities in **I** prepared by reaction with degraded DNP. Column: 5-μm Kromasil C$_8$ (25 cm × 4.6 mm I.D.). Mobile phase A: 0.05 M ammonium acetate, pH 2.5. Mobile phase B: methanol–acetonitrile (30:70). Gradient program: 16% B (16 min) then 16% B to 80% B (20 min). Detection: 250 nm. Flow-rate: 1.0 ml/min.

Linearity of response

The linearity of response of **IV** was determined. A stock solution of the reference standard of **IV** (approximately 500 μg per ml) was prepared and serially diluted to give five solutions with a concentration range between 0.5 and 5.0 μg/ml. The solutions were chromatographed according to the meth-

od. A plot of area *versus* concentration of **IV** gave a straight line. The least squares fit of the data gave the equation

$$\text{area} = (3.784 \pm 0.009) \cdot 10^4 \cdot \text{concentration } (\mu\text{g/ml}) + (31 \pm 260)$$

The correlation coefficient is 1.000. The intercept is not significant.

Peak homogeneity

The purity of the chromatographic peak of **IV** was determined by diode array spectroscopy. A sample of DNP was reacted and chromatographed according to the method. The UV spectrum of the eluent from the chromatographic run was recorded. Analysis of the chromatographic peak of **IV** showed it to be pure with respect to other components with different ultraviolet spectra.

Limit of detection

The limit of detection for the method was determined from a chromatogram of a standard **IV** solution. The limit of detection defined as the concentration of analyte that gives a peak height equal to three times the baseline noise was determined as 0.02 μg/ml.

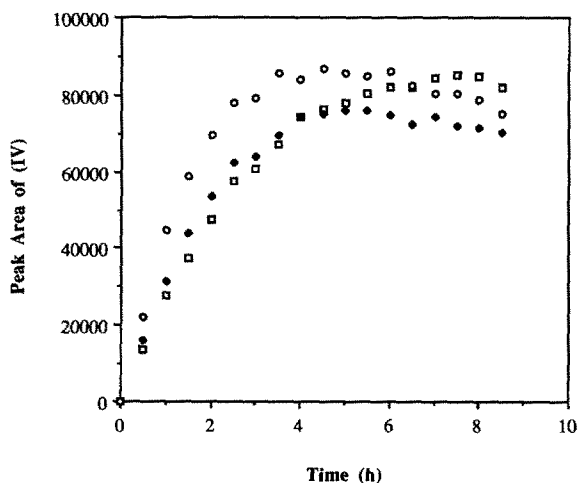Fig. 4. Kinetic profile of oxindole reaction at 30 (□), 35 (◆) and 40°C. (○).

Precision

The reproducibility of the procedure was tested by performing five determinations over two separate days on the same DNP sample. For each determination the reaction with oxindole was performed and the amount of **IV** produced was determined. The results for day one in units of μg **IV** produced/g DNP were 144.4, 152.0, 153.7, 152.1, and 154.4; for day two, they were 142.2, 153.1, 149.0, 145.8, and 149.9. The mean for day one was 151.3 with a standard deviation of 4.0; the mean for day two is 148.0 with a standard deviation of 4.1. There is no significant difference between the means of the two days ($p = 0.05$).

Correlation data

The five samples of DNP of varying quality were reacted with oxindole. The same samples of DNP were reacted with **II** at the same time. The products were analysed according to the methods described here. The amount of **IV** formed in the reaction with oxindole was directly proportional to the purity of the DNP. A plot of the data is shown in Fig. 5. The equation of the least squares fit of the data is

μg **IV** formed/g DNP $= (7.55 \pm 0.34) \cdot$ purity $+ (57 \pm 21)$

where purity is expressed as % impure DNP in reagent. The correlation coefficient is 0.994. The amount of **VI** formed in the reaction with **II** also
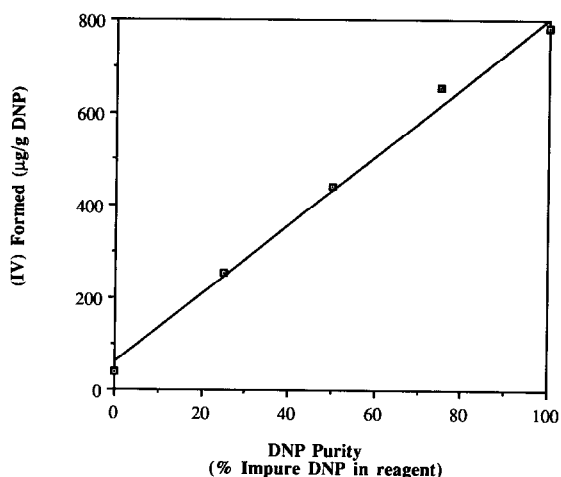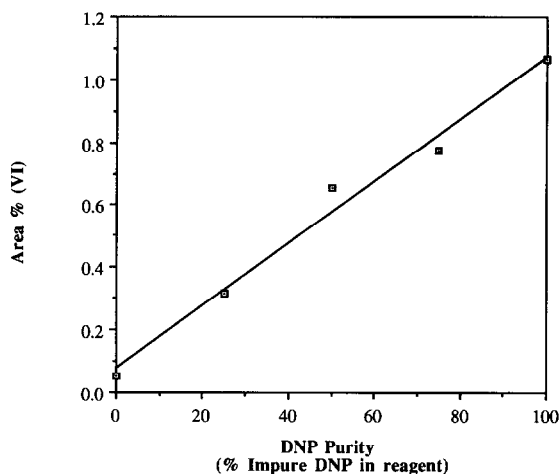

Fig. 6. Plot of **VI** formed from the reaction of **II** with DNP of varying purity. The concentration of **VI** is expressed as a peak area ratio relative to the peak area for **I**.

was directly proportional to the purity of the DNP. A plot of the data is shown in Fig. 6. The equation of the least squares fit of the data is

area % **VI** $= (9.94 \pm 0.72) \cdot 10^{-3} \cdot$ purity $+ (0.076 \pm 0.044)$

where purity is expressed as % impure DNP in reagent. The correlation coefficient is 0.985. Since the two sets of data were generated from the same five DNP samples, one set of data may be plotted against the other. Fig. 7 shows area % of **VI** plotted
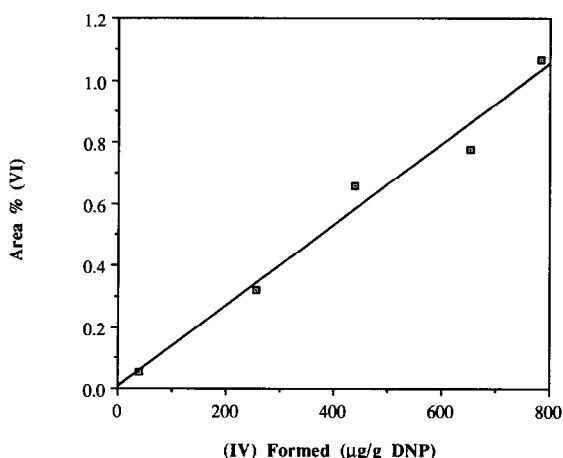

Fig. 5. Plot of **IV** formed from the reaction of oxindole with DNP of varying purity.

Fig. 7. Correlation of compounds **IV** formed to area % **VI** for reaction with DNP of varying purity.

as a function of μg **IV** formed/g DNP. The least squares fit of the data is

area % of **VI** = $(1.31 \pm 0.12) \cdot 10^{-3} \cdot \mu$g **IV** formed/g DNP + (0.006 ± 0.060)

The correlation coefficient is 0.976.

A specification for DNP, in terms of **IV** formed from the oxindole reaction, was established using Fig. 7. DNP not satisfying the specification could thus be rejected for use in the synthesis of ropinirole. DNP should be used as soon as practicable after testing, since DNP will degrade upon exposure to air.

CONCLUSION

An unknown trace impurity or impurities in DNP lead to the formation of an unwanted impurity in the drug substance ropinirole. A simple model reaction of oxindole with impurities in DNP was shown to produce a product analogous to the impurity in ropinirole and the kinetics of the model reaction were optimised. An HPLC procedure to quantitate the products of the model reaction was developed and validated. The method was shown to be linear over the concentration range of interest, specific and sensitive. The amount of product in the model reaction was shown to correlate with the impurity in ropinirole to levels below 0.1% when the same batch of DNP was used in both reactions. Therefore, the model reaction and the corresponding HPLC procedure can be used to determine the acceptability of DNP for use in the ropinirole synthesis.

REFERENCES

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2 G. Tacconi, *J. Chem. Soc., Perkin Trans 2*, (1976) 150.
3 A. Wahl and P. Bagard, *Bull. Soc. Chim. Fr.*, 5 (1909) 1033.