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COMMUNICATIONS

LC–MS Identification of the Degradates of NGP1-01 and Subsequent Development of a Routine Stability-Indicating Method of Analysis

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ABSTRACT

NGP1-01 (1) is a heterocyclic cage compound with calcium channel blocking activity similar to that of nifedipine. Since no data on the stability or mechanism of degradation of this type of compound are available, the kinetics of degradation and pH stability were investigated. The degradates formed at pH 2, 5, and 9 were separated and identified with LC-MS. Chromatography was performed on a Hypersil phenyl column, 150 × 2.1 mm, flow 0.2 ml/min, mobile phase acetonitrile–0.05 M ammonium acetate pH 4.7, 30/70 for 5 min, then gradient to 65/35 at 10 min. The MS was operated in the electron ionization mode at 70 eV. It was found that pH did not have an influence on the mechanism of degradation, and the same degradates were observed throughout the pH range investigated, namely benzylamine and a heterocyclic keto-alcohol. A method for routine stability-indicating analysis of (1) was developed using a phenyl column (5 μm, 250 × 4.6 mm Hypersil) with a mobile phase of acetonitrile–0.01 M ammonium phosphate, 0.3% triethylamine, pH 4.5 60/40. Flow rate was 1 ml/min, injection volume 20 μl, and UV detection at 210 nm. The method proved usable over a range of 15–1000 μg/ml, with an accuracy of 100.1 ± 0.55% and precision of <2% variance over the stated range. Reliable kinetic studies could be conducted by using this method of analysis.

INTRODUCTION

NGP1-01, 8-benzylamino-8,11-oxapentacyclo[5.4.-$0.0^{2,6}.0^{3,10}.0^{5,9}$] undecane (**1**, Fig. 1) is a heterocyclic cage compound with calcium channel blocking activity similar to that of nifedipine (1). The chemical structure of **1** was elucidated by Marchand et al. (2), who published the IR, ^{1}H- and ^{13}C-NMR spectra; and by van der Schyf et al. (3), who used NMR spectroscopy and x-ray crystallography. No data on the stability or mechanism of degradation of this type of compound is available. Liquid chromatography-mass spectroscopy (LC-MS) was used to identify the degradates, and a reliable stability-indicating method of analysis was developed to study the kinetics of degradation and pH stability.

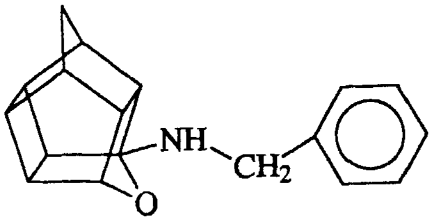
EXPERIMENTAL

Chemicals and Reagents

NGP1-01 was synthesized in this laboratory (4-6). Heptacosafluorotributylamine was supplied by Aldrich (Milwaukee, WI). All other chemicals were analytical grade, and solvents were high-performance liquid chromatography (HPLC) grade (E. Merck S. A. (Pty) Ltd.).

Samples

NGP1-01 (0.02 M solution) was decomposed in glycine buffer pH 2 and 9, and in acetic acid/sodium acetate buffer pH 5. All buffers were of ionic strength 0.1. The solutions were kept in an oven at 50°C in glass reaction vials purged with nitrogen. Samples were filtered through 0.45-µm hollow fiber syringe filters (Dynagard®, Microgon, Laguna Hills, CA) prior to analysis.



(1)

Figure 1.

Apparatus and Operating Conditions

The LC system used was a Hewlett Packard 1090 ternary gradient system equipped with a diode array UV detector and Pascal Chemstation software.

The HPLC was coupled to a Hewlett Packard 59980B particle beam LC-MS interface and a HP 5989A mass spectrometer. The MS was operated in the electron ionization mode at 70 eV, positive polarity with a source temperature of 250°C, and a probe temperature of 100°C. Spectra were recorded over an *m/z* range of 45-350 at 1.5 sec/scan. The instrument conditions were optimized daily with the mobile phase before commencement of analyses. Heptacosafluorotributylamine was used to calibrate the instrument.

HPLC-MS

For LC-MS a Hypersil phenyl microbore column, 3 µm particle size, 150 mm × 2.1 mm (Phenomenex, Torrance, CA) was used with a mobile phase of acetonitrile-0.01 M ammonium acetate and 0.1% triethylamine in water, pH 4.7. The acetonitrile concentration was kept constant at 30% for the first 5 min, followed by a linear gradient to 65% after 10 min. The flow rate was 0.2 ml/min, and 10 µl was injected. The effluent was monitored by means of a diode array detector at 210 nm before passing into the MS.

HPLC-Diode Array Detection

For routine quantitative analysis a Hypersil phenyl column (5 µm, 250 mm × 4.6 mm, Phenomenex) was used with a mobile phase of acetonitrile-0.01 M dibasic ammonium phosphate buffer with 0.3% triethylamine, adjusted to a pH of 4.50 with phosphoric acid (60:40, v/v). The flow rate was 1 ml/min, injection volume 20 µl and detection was ultraviolet (UV) at 210 nm.

RESULTS AND DISCUSSION

Identification of Degradatess by Liquid Chromatography-Mass Spectrometry

The chromatograms of the pH 2, 5, and 9 samples exhibited exactly the same peaks, although the relative peak heights varied, suggesting that pH influenced the rate but not the mechanism of degradation. These pHs were chosen to obtain stability data on the ionized and un-ionized molecule (pK_a of **1** is 7.07), as well as the influence of acids and bases on stability. The mass spectrum obtained for **1** [Fig. 2(a)] corresponded well with previous spectra obtained for the pure compound. Benzylamine (**3**) was identified by means of a library search (95% match) as well as by comparison to the spectrum of a standard. The compound eluting after 4.7 min has an *m/z* of 176, which corresponds to [M^+] of the keto-alcohol (**2**) in Fig. 2(b).

HPLC with Diode Array Detection

The diode array detection (DAD) spectra of the three compounds in the two mobile phases were compared to see if DAD could be used to identify the analytes in the mobile phase developed for routine analysis. Figure 3 shows that the spectra obtained were comparable although the mobile phase differed. A library search without retention window was able to positively identify the three analytes.

The method described for routine stability-indicating analysis was optimized for acetonitrile and triethylamine content as well as pH. Diode array spectra could be used to identify **1, 2,** and **3** by comparison to spectra stored in library, and peak purity of the eluted peaks could also be determined. The accuracy over the range 50–1000 μg/ml was 100.05%, and the precision was ±0.82%. The limit of detection for **1** was 0.1 μg/ml (peak 3 times baseline noise).

CONCLUSION

HPLC with DAD proved useful in routine stability-indicating analysis of **1** after the degradates were first identified with LC–MS.

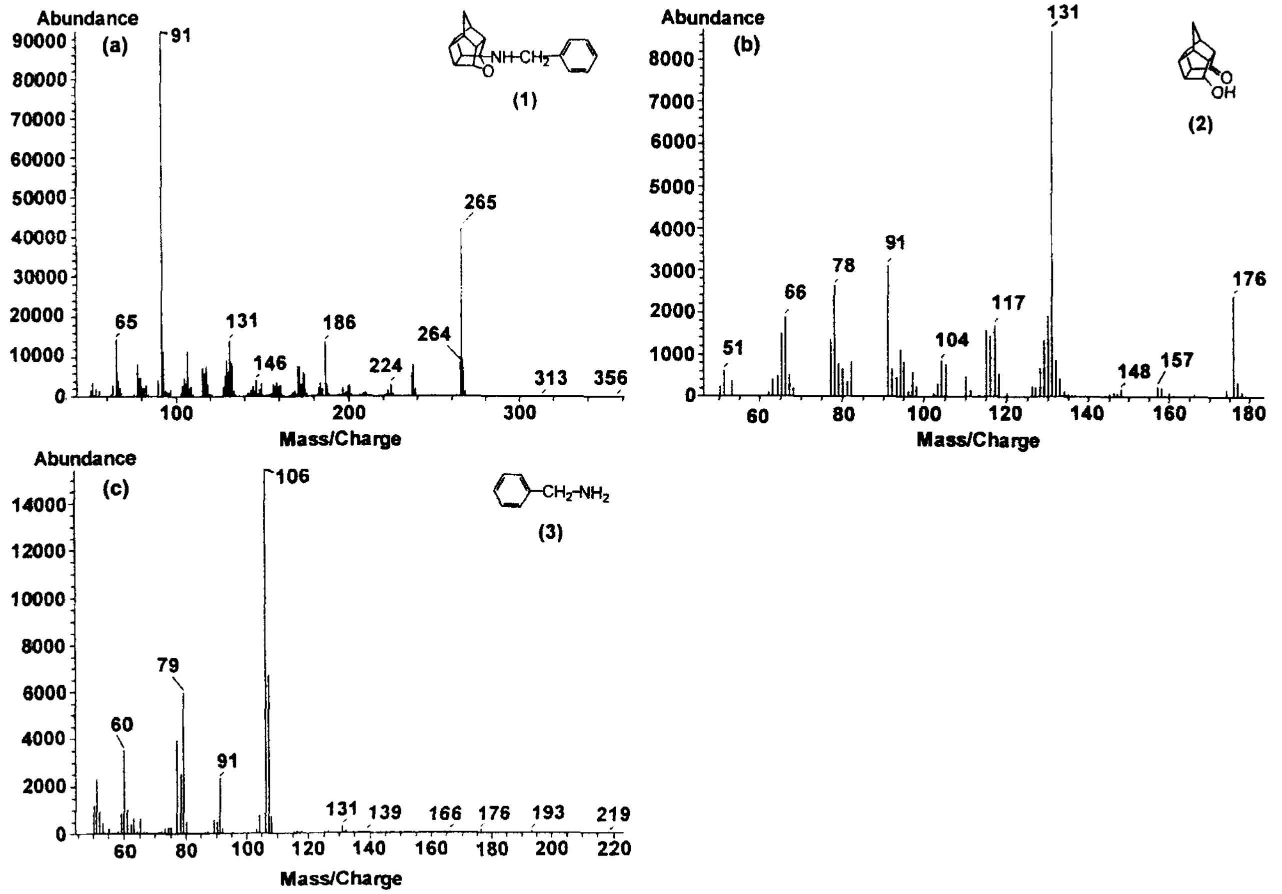


Figure 2. Mass spectra of (a) NGP1-01, (b) keto-alcohol degradate, and (c) benzylamine degradate.

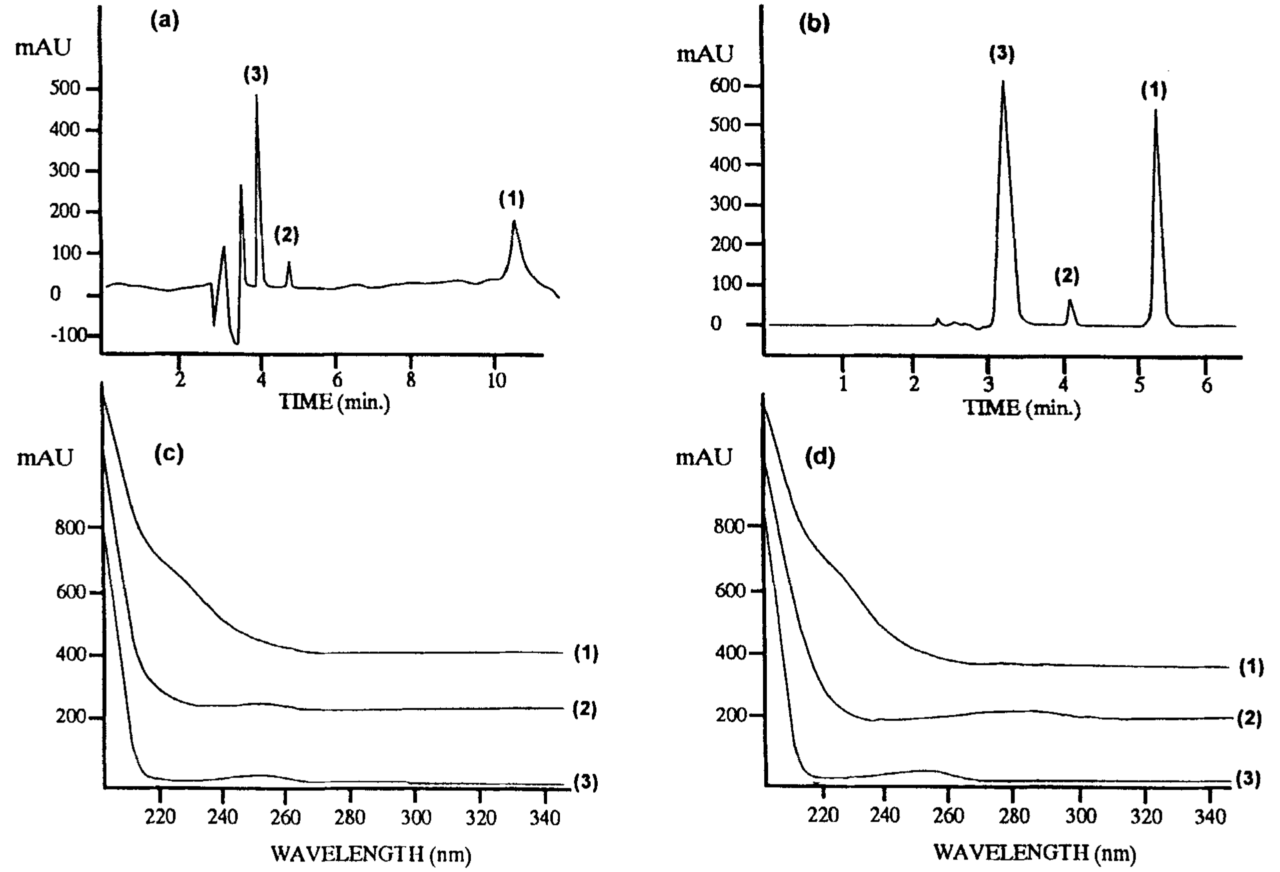


Figure 3. HPLC chromatograms of (a) the LC-MS separation, and (b) the routine stability-indicating method; and the DAD spectra obtained in (c) the LC-MS and (d) the stability mobile phase.

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