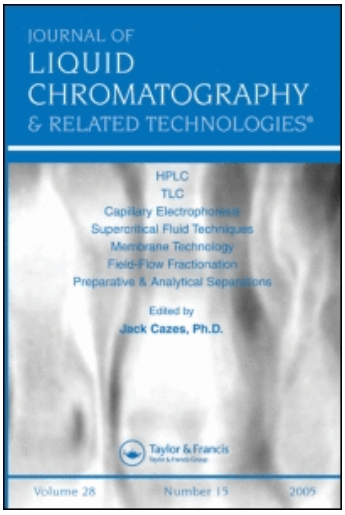


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# Determination of N-Vinyl-2-Pyrrolidinone (NVP) in Rat and Dog Plasma by High-Performance Liquid Chromatography

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# DETERMINATION OF N-VINYL-2-PYRROLIDINONE (NVP) IN RAT AND DOG PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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**ABSTRACT**

A method for the determination of N-vinyl-2-pyrrolidinone (NVP) in both rat and dog plasma is described. The method involves the addition of an internal standard, a solution containing sodium lauryl sulfate, and an ultrafiltration step through a micropartition system which is subjected to centrifugation at 2000 x g for 15 minutes. The ultrafiltrate is then analyzed by an HPLC system with UV detection at 235 nm. The assay is linear in the concentration range of (0.2-8.0 μg/mL) for rat plasma with a minimum sensitivity of 100 ng/mL and a day to day variation of 3.0%. In the case of

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dog plasma, the assay is linear in the concentration range of (0.05-4.0 μg/mL) with a minimum sensitivity of 50 ng/mL when 100 μL aliquots of plasma are extracted. Application of this method to the protein binding studies of NVP to both rat and dog plasma is illustrated.

## INTRODUCTION

N-vinyl-2-pyrrolidinone (NVP) is used as a chemical precursor in the production of copolymers and the manufacture of its homopolymer, polyvinyl-pyrrolidinone (PVP)(1).

PVP has several industrial applications due to its film-forming and adhesive properties, its unique colloidal and dispersing abilities, and its capacity to form complexes with certain compounds. Due to these characteristics, PVP enjoys wide usage in the cosmetic, pharmaceutical, plastic, beverage, and other industries(2).

PVP preparations employed in all of the aforementioned applications contain a small percentage of NVP as an impurity. The U.S. Pharmacopeia XXII permits the presence of up to 0.2% w/w of NVP in PVP (Povidone, USP)(3). This percentage represents a limit of NVP concentration in PVP, the actual amount of NVP present in PVP is usually lower.

The present communication describes an analytical method for the quantitation of NVP in rat and dog plasma. The procedure involves an ultrafiltration extraction followed by HPLC analysis. The assay was applied to a study of protein binding of NVP to both rat and dog plasma.

## EXPERIMENTAL

### Materials.

N-vinyl-2-pyrrolidinone (NVP) was purchased from Aldrich Chemical Company (Milwaukee, WI). The internal standard, acetanilide, was obtained from the J.T. Chemical Company (Phillipsburg, NJ). N-[$^{14}$C-vinyl]-2-pyrrolidinone with a specific activity of 1.7 mCi/mmole was purchased from Sigma Laboratories, Inc. (St. Louis, MO). Scintiverse LC and sodium phosphate dibasic heptahydrate were acquired from Fisher Scientific (Fairlawn, NJ). Sodium lauryl sulfate was obtained from Sigma Chemical Company (St. Louis, MO). HPLC grade acetonitrile and methanol were purchased from Fisher Scientific. All water used in this assay was double distilled, treated by a NANOpure II water purification system (SYBRON, Boston, MA), and subsequently filtered through a 0.45-$\mu$m pore Nylon filter.

High-Performance Liquid Chromatography Equipment.

The HPLC system consisted of an Altex (Berkley, CA) model 110A solvent metering pump, a Rheodyne (Cotati, CA) sample injector equipped with a 100 μl injector loop, and a Kratos (Ramsey, NJ) Spectroflow 773 variable UV detector. The detector output was connected to a Linear Instruments (Reno, NV) model 500 dual channel chart recorder.

In the case of $^{14}C$-containing samples, a radiomonitored HPLC system was utilized. This system included the aforementioned pump, detector, and chart recorder which were appropriately connected to a Packard Instruments (Downers Grove, IL) HS Flo-1 radioactive flow through detector. The radioactive detector was equipped with a Radiomatic Instruments (Tampa, FL) model ES stream splitter. The mixing ratio of scintillation cocktail (Radiomatic Flo-Scint II) to column effluent was 4:1 v/v. The output of the radioactive flow through detector was recorded simultaneously on the second channel of the recorder.

Chromatographic Conditions.

The mobile phase consisted of water:methanol (80/20, v/v) which was deaerated under vacuum. The

mobile phase was filtered through a nylon filter (0.45 μm) prior to use. The flow rate was 1.0 mL/min through a μ-Bondapak C18 column (10-μm particle diameter, 3.9 x 300 mm, Waters Associates, Milford, MA) protected by a C18 pellicular packing guard column (Direct-Connect Guard Column, Alltech-Applied Science, Deerfield,IL). The column was maintained at room temperature. Injection volumes were 50 to 100 μL. The ultraviolet detector was set at 235 nm with a sensitivity of 0.025 AUFS. The peaks were recorded with a 5-10 mV recorder at a chart speed of 2.0 cm/min.



**Preparation of NVP Standard and Stock Solutions.**

A stock solution (5.0 mg/mL) of NVP in double distilled water (pH = 6.8) was found to be stable for at least two months at 4°C. This observation is found to agree with work which was reported previously from this laboratory(4). Standard solutions containing between 2.0 μg/mL and 40.0 μg/mL of NVP were prepared by diluting the stock solution with double distilled water.

Plasma standards were prepared by diluting the NVP stock solution to 40.0 μg/mL with drug-free plasma. This solution was further diluted with

drug-free plasma to yield standard plasma samples containing NVP in concentrations ranging from 0.05 to 8.0 μg/mL.

An acetanilide stock solution containing 160 μg/mL in double distilled water was shown to be stable for at least one month at 4°C. From this stock solution, the working internal standard solutions of 4 μg/mL were freshly prepared on each day of analysis by appropriate dilution with double distilled water.

A solution of sodium lauryl sulfate (0.5%, w/v) was made by dissolving sodium lauryl sulfate in a solvent (pH = 7.4) consisting of 35% methanol; 65% phosphate buffer (v/v) in double distilled water.



Plasma Extraction Procedure Using Ultrafiltration.

A 50 μL aliquot of the internal standard solution of acetanilide (200 ng) and a 100 μL aliquot of the 0.5% sodium lauryl sulfate solution were added to a 100 μL aliquot of plasma sample. This solution was gently mixed, transferred to an AMICON (Danvers, MA) micropartition system (MPS-1) equipped with a YMT membrane (14 mm, Amicon), and centrifuged (2000 x g) for 15 minutes.

Subsequently, a 20-60 μL aliquot of the ultrafiltrate was injected into the HPLC system which was described earlier.

NVP Concentration Determination.

An 8-point plasma standard curve was constructed by plotting the peak height ratio of NVP to the internal standard versus plasma standard concentration. The NVP standard concentrations were 0.2, 0.4, 0.6, 0.8, 2.0, 4.0, 6.0, and 8.0 μg/mL. The data from the standard curve were analyzed by linear regression and the resulting equation was used to calculate the concentration of NVP in the rat plasma samples.

For the dog plasma samples, an 8-point plasma standard curve was constructed in the same manner as was done for the rat plasma samples. The NVP standard concentrations were 0.05, 0.1, 0.15, 0.2, 0.5, 1.0, 2.0, and 4.0 μg/mL. Again, the data from this curve were analyzed by linear regression, and the equation was used to determine NVP concentrations. In addition to the standard curve, a one point standard was run daily with each series of plasma samples. This standard was used as a device for checking the reproducibility of the HPLC method.

Determination of Extraction Efficiency.

Plasma samples containing 0.4 μg/mL and 4.0 μg/mL NVP were extracted using the previously described ultrafiltration procedure. Extraction efficiency was calculated by comparing the amount of NVP in the extracted sample to that of contained in an equal volume of unextracted plasma.

Binding of NVP to Plasma Proteins in the Rat and the Dog.

The in vitro protein binding studies were carried out at two concentrations of NVP (1 μg/mL and 10 μg/mL) for both the rat and dog plasma samples. To a 200 μL aliquot of plasma containing NVP, a 20 μL aliquot of a 10μg/mL acetanilide stock solution was added. This mixture was then gently mixed and subjected to ultrafiltration (AMICON MPS-1 system) at 2000 x g for 15 minutes. The concentration of NVP present as a free fraction was determined by analysis of the ultrafiltrate by HPLC. The total fraction of NVP present in the plasma sample was determined by following the same work up on another plasma sample at the same NVP concentration. Instead, however, a 100 μL aliquot of the 0.5% sodium lauryl sulfate solution was

added to the plasma sample prior to ultrafiltration.

The *in vivo* protein binding studies were carried out on plasma that had been taken during either an oral or an iv dosing study. After being analyzed for NVP by the extraction method described earlier, the same procedures were followed for these samples as was followed for the *in vitro* binding studies.

The extent of binding of NVP to plasma proteins was calculated by the following equation:

$$\% \text{ Bound} = \frac{\text{Total NVP fraction} - \text{Free NVP fraction}}{\text{Total NVP fraction}} \times 100$$



**Determination of $^{14}$C-NVP in Plasma.**

$^{14}$C-NVP plasma concentrations were determined by utilizing the HPLC radiomonitored system described earlier and by following the previously published techniques from this laboratory(5).

## RESULTS AND DISCUSSION

Attempts to recover $^{14}$C-NVP from plasma samples by precipitation of their protein fraction with concomitant extraction of NVP with methanol

were unsuccessful. Thus, when the methanol extracts were evaporated under nitrogen and subsequently examined by radiomonitored HPLC, the recovery of the $^{14}$C-NVP from plasma was found to be 12.3 ± 2.2%. Similarly, low recovery values of NVP (1.9 ± 3.0%) were obtained by methanolic extraction of aqueous solutions (pH = 7.2) of NVP. Each of these recovery values represents the mean from at least three determinations. Similar results were obtained when methylene chloride was used as the extraction solvent.



Although NVP has been reported to decompose in acid media(6), the compound is stable at pH 7.2 ($t_{\frac{1}{2}}$ > 24h)(4). Thus, pH does not appear to be a major contributing factor in its poor recovery from aqueous media. Therefore, the poor recovery of $^{14}$C-NVP from aqueous solutions suggested that NVP was probably volatilized during the evaporation of the extraction solvent. It was therefore concluded that separation of NVP from plasma by ultrafiltration was a necessary step in the analysis of NVP in biological samples.

The percent of binding of NVP to plasma proteins was found to range from 9-13% (Table 1). These values were obtained with two plasma

**TABLE 1.** Percent Binding of NVP to Plasma Proteins in Rat and Dog

| | Total NVP Plasma Concentration (μg/mL) | % Binding Rat | % Binding Dog |
|---|---|---|---|
| In Vitro[a] | 1.0 | 9.0 ± 1.7 | 8.5 ± 3.3 |
| | 10.0 | 7.1 ± 1.3 | 8.6 ± 1.1 |
| In Vivo[b] | 1.1 ± 0.2 | 10.5 ± 0.9 | - |
| | 1.0 ± 0.3 | - | 12.6 ± 7.8 |
| | 11.4 ± 0.8 | - | 9.7 ± 5.8 |

[a] Mean ± SD (n=3).

[b] Values have been obtained from the use of one animal and represent the mean ± SD of three repeated analysis trials.



concentrations of NVP utilizing plasma from both rat and dog. The extent of protein binding was assessed in plasma samples spiked with known concentrations of NVP, or with samples of plasma obtained freshly from live animals to which NVP was administered by oral or iv routes.

From the results shown in Table 1, it became clear that the addition of sodium lauryl sulfate prior to the ultrafiltration of plasma samples was an important step for the dissociation of their protein-bound fraction of NVP.

Figure 1 shows chromatograms of rat plasma with no NVP added, rat plasma spiked with NVP, and rat plasma after oral administration of 0.5 mg/kg of NVP. Under the chromatographic conditions specified in this method, the retention times for NVP and the internal standard were found to be 11.5 and 15.5 min, respectively. No endogenous plasma components or metabolites of NVP were observed near the NVP peak or the internal standard peak. The detection limit of the assay was determined to be approximately 0.1 μg/mL in rat plasma with a sample volume of 100 μL (Figure 2A). NVP concentration and peak height ratio of NVP to internal standard were found to be linearly related throughout the NVP concentration range investigated (0.2-8.0 μg/mL) (Figure 2A). Linear regression analysis produced an equation of $y = 0.651x - .003$ with a correlation coefficient of 1.000.

In the case of dog plasma, the detection limit of the assay was determined to be 0.05 μg/mL with a sample volume of 100 μL (Figure 2B). Again, the standard curve showed linearity through the concentration range investigated (0.05-4.0 μg/mL) (Figure 2B). Linear regression analysis produced an equation that was similar to the standard curve

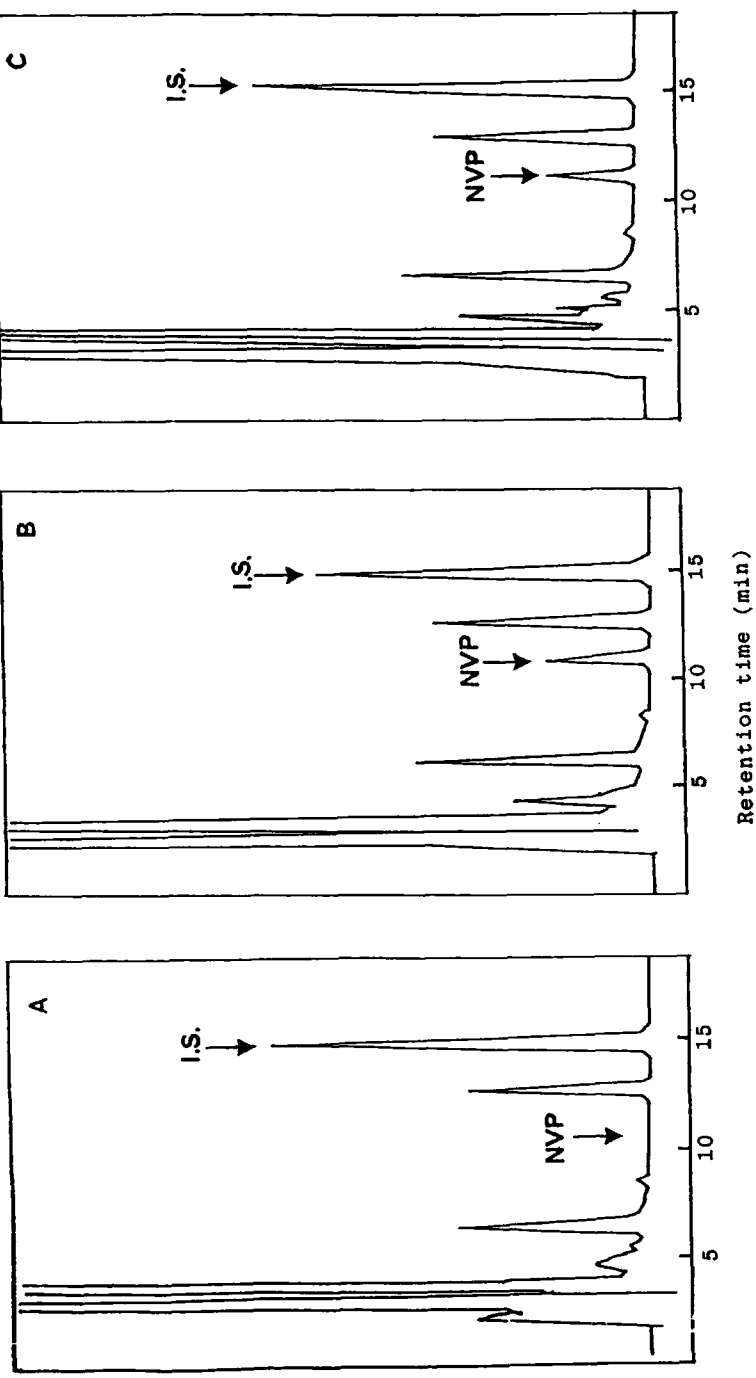


Figure 1-Chromatograms of (A) blank rat plasma, (B) rat plasma spiked with NVP at a concentration of 0.4 μg/mL, and (C) rat plasma taken one hour after receiving an oral dose of 5 mg/kg NVP (I.S. = internal standard).

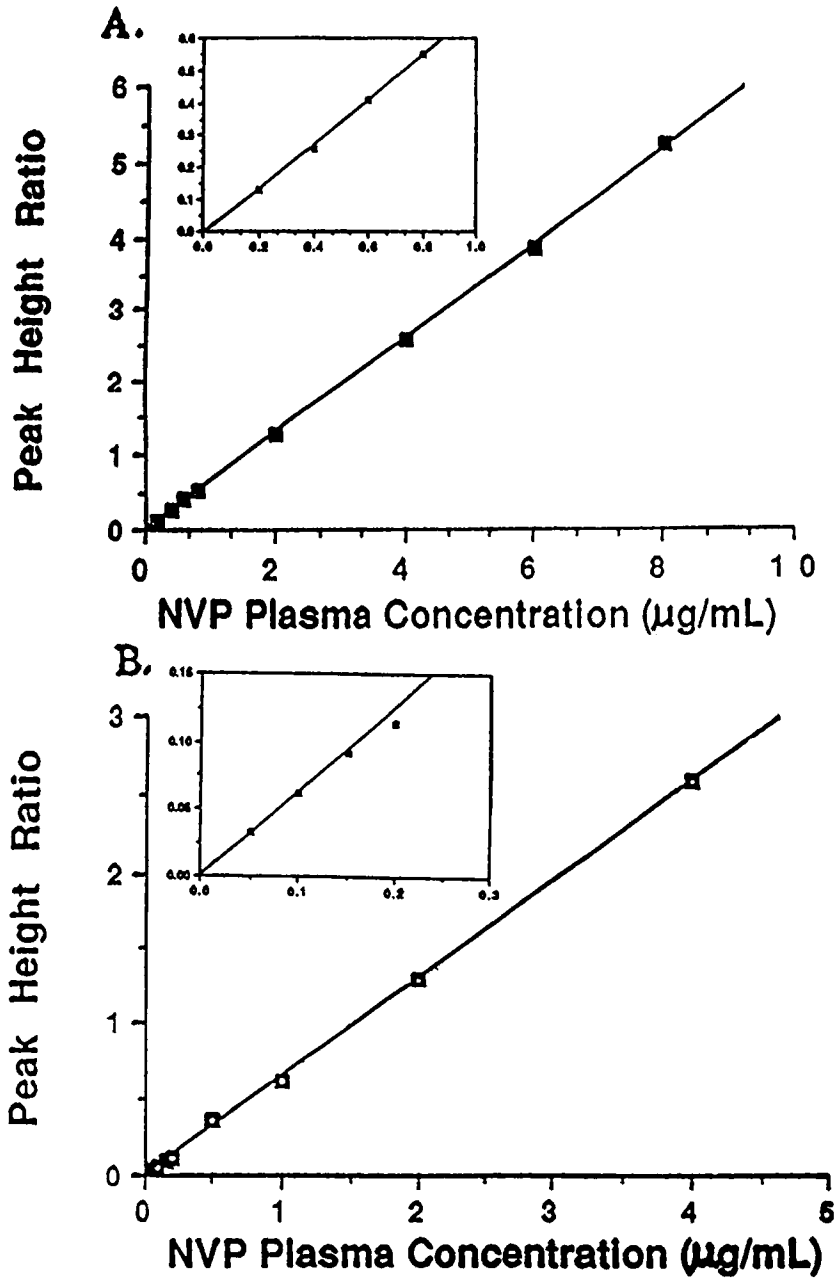


Figure 2-Standard curves for the HPLC analysis of NVP in (A) rat plasma and (B) dog plasma (Range of A = 0.2 to 8.0 µg/mL; range of B = 0.05 to 4.0 µg/mL). The enlarged portions of each curve are to show the fit of the curve and the low concentrations of NVP.

**TABLE 2.** Extraction Efficiency and Variation of the Determination of NVP in Rat Plasma.

| Spiked NVP Plasma Concentration (μg/mL) | [a] NVP Extraction Efficiency (%) | [b] Coefficient of Variation (%) Intraday | Interday |
|---|---|---|---|
| 0.4 | 99.0 ± 3.3 | 1.8 | 3.0 |
| 4.0 | 96.8 ± 1.5 | 2.4 | 1.2 |

[a] Mean ± SD (n=4).

[b] Based on quadruplicate determinations on each of 3 days; mean ± SD.



which was constructed from rat plasma. The equation for the dog plasma standard curve was $y = 0.647x + .003$ with a correlation coefficient of 0.999.

The extraction efficiency of NVP using this analytical procedure was found to range from 99.0 ± 3.3% and 96.8 ± 1.5% at 0.4 and 4.0 μg/mL of NVP in rat plasma, respectively (Table 2).

Intraday variation of the extraction method was determined from replicate analysis (n = 4) of two spiked plasma standards at concentrations within the linear range of the assay (Table 2). The intraday deviation of NVP recovery was found to range from 1.8 to 2.4%. Interday deviation of

recovery of NVP was found to range from 1.2 to 3.0% (Table 2).

The assay of NVP reported in this communication represents a rapid and convenient procedure for the quantitation of NVP in biological fluids. Ongoing studies in these laboratories have shown that this assay is useful in conducting pharmacokinetics studies with NVP. Plasma concentrations of intact NVP after administered iv (0.5 and 5.0 mg/kg) and oral (5.0, 10.0, and 20.0 mg/kg) doses of the monomer in both the rat and dog are currently being analyzed by this assay. The results of these studies will be presented in a future publication.



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