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# Stability of the tranquilizer drug propionylpromazine hydrochloride in formulated products<sup>†</sup>

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**Abstract:** An analytical method to evaluate propionylpromazine hydrochloride (PPZHCl) in tranquilizer formulations was developed using high-performance liquid chromatography (HPLC). During analysis of aged quality-control samples, a previously unreported chromatographic response was observed at a shorter retention time than PPZHCl. Further investigation of formulations stored in trap tap devices at temperatures ranging from 5 to 40 °C during field trials at four different locations confirmed the degradation of the active ingredient. Further investigation using HPLC/tandem mass spectrometry revealed two to five degradates, with the major degradates being oxidation products of the active ingredient, PPZHCl. As PPZHCl formulations must be stable when stored at 5 to 40 °C for 6 to 12 months, reformulation with the anti-oxidant ascorbic acid was utilized to achieve the required PPZHCl stability. Published in 2005 for SCI by John Wiley & Sons, Ltd.

**Keywords:** propionylpromazine hydrochloride; degradates; liquid chromatography; HPLC/mass spectrometry

## 1 INTRODUCTION

1-{10-[3-(Dimethylamino)propyl]phenothiazin-2-yl}propan-1-one hydrochloride (propiomazine hydrochloride; propionylpromazine hydrochloride; PPZHCl) is used by cattle and swine producers to reduce animal stress during transportation from farms to slaughterhouses.<sup>1</sup> Commercially available Tranvet<sup>®</sup> tablets containing PPZHCl are used by veterinarians to sedate dogs. To evaluate PPZHCl residue levels in swine tissues, a number of chromatographic methods including high-performance liquid chromatography,<sup>1</sup> gas chromatography<sup>2</sup> and thin layer chromatography<sup>3,4</sup> have been developed. Recently, the United States Department of Agriculture (USDA) evaluated the use of PPZHCl with foothold traps to reduce post-capture trauma. Such traps are frequently used by the Wildlife Services program to capture coyotes that prey on livestock. In efforts to release itself from the trap, the animal itself may inflict other types of trauma including broken teeth, tongue and gum lacerations<sup>5</sup> along with chewing and gnawing of the restricted appendage.<sup>6</sup> Incorporating a tranquilizer delivery mechanism with a leghold trap may reduce the amount of self-inflicted trauma on a restrained animal.

Balser<sup>6</sup> tested such a device by attaching a chewable apparatus consisting of semi-rotten cloth, petroleum

jelly and the sedative diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one), sealed with a beeswax:paraffin mixture, to a leghold trap. The idea was that a restrained animal, in an effort to free itself, would chew on this tranquilizer trap device (TTD) and ingest the sedative. Balser<sup>6</sup> observed that captive animals chewed on the delivery device. Diazepam ingestion caused drowsiness and the reduction or absence of biting which reduced the number of self-inflicted wounds. However, diazepam being on the Drug Enforcement Administration list of controlled substances was never authorized for use with TTDs. Laboratory experiments<sup>7</sup> and field studies<sup>8</sup> found PPZHCl to be a favorable substitute for diazepam. Linhart *et al*<sup>8</sup> noted that, during a 24-h check period, 86% of captive animals restrained by control traps not equipped with a tranquilizer device exhibited some type of foot damage, while only 10–25% of the animals caught in leghold traps equipped with PPZHCl containing TTDs exhibited foot damage.

To aid Wildlife Services in the development of TTDs, the National Wildlife Research Center (NWRC) obtained an Investigational New Animal Drug Application (INADA) from the Food and Drug Administration for PPZHCl. Two types of TTD

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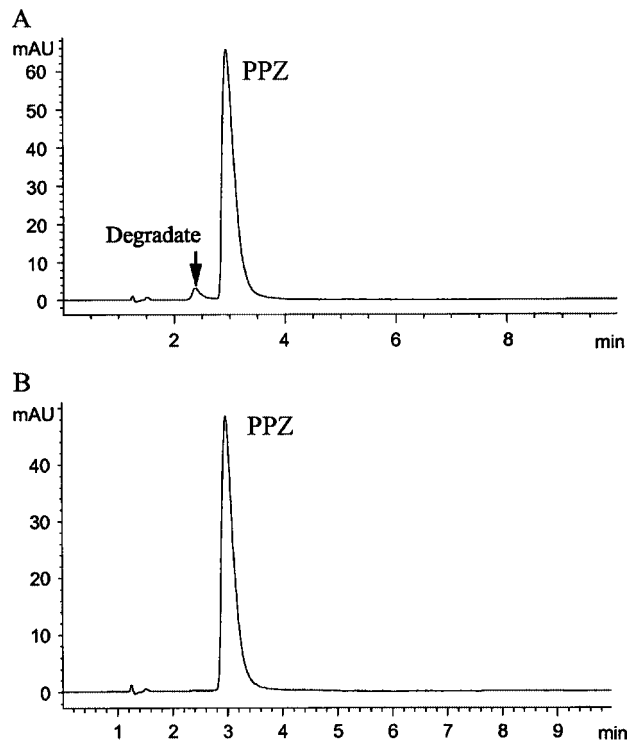
Contract/grant sponsor: National Wildlife Research Center

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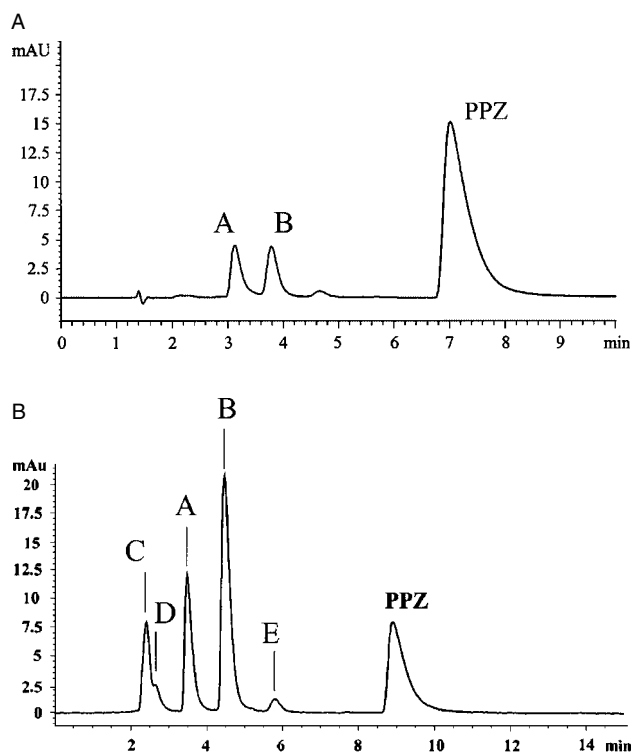
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evaluated by Wildlife Services included the dart balloon and the McBride Device (Livestock Protection Company, Alpine TX). Each TTD contained 600 mg of PPZHCl combined with one of three matrices including K-Y Jelly®, Karo® dark corn syrup or Vaseline®. After evaluation of all the devices, the K-Y Jelly formulation was chosen, but the formulation was packaged in the newer ‘pipette’ device. This was a very serviceable and well manufactured device that was much easier and cheaper to produce than the McBride and balloon devices (BlomS, pers comm).

An analytical method to evaluate the PPZHCl content of K-Y Jelly formulations was developed using ion-pairing HPLC.<sup>9</sup> During this work, a previously unreported chromatographic response was observed in a sample that had been stored at ambient temperatures for approximately a month (Fig 1). Higher concentrations of this same compound were observed in older samples (Fig 2), as well as a reduction in the quantity of active ingredient. TTDs from field trials at four different locations (Guam, Utah, Idaho and Minnesota) confirmed the degradation of the active ingredient under storage conditions at temperatures ranging from 5 to 40 °C. Analysis of samples stored at 20–25 °C over several months indicated that PPZHCl degraded by approximately 3% per month with an even higher rate of degradation at 40 °C. As K-Y Jelly had optimal handling and delivery characteristics, we attempted to: (1) determine the identity of the degradation products, (2) elucidate the mechanism of PPZHCl degradation and (3) identify



**Figure 1.** Chromatograms of PPZHCl formulations (A) with and (B) without degradation observed in the ion-pairing chromatographic system.



**Figure 2.** Chromatogram of degradates with 1% acetic acid in 60 + 40 methanol + water mobile phase: (A) TTD device stored at ambient for three months; (B) TTD device stored at ambient for seven months.

an additive which would improve the stability of PPZHCl in K-Y Jelly formulations under field conditions.

**2 EXPERIMENTAL**

**2.1 Preparation of in-house formulations**

For each PPZHCl formulation, three replicates were prepared by: (1) recording the weight of an empty 10-ml graduated centrifuge tube, (2) drawing up the control matrix into a glass pipette and dispensing the appropriate volume into the pre-weighed tube and recording the weight, (3) adding the specified amount of PPZHCl technical material to the centrifuge tube, recording the final weight and mixing with a spatula until the active ingredient was uniformly dispersed throughout the matrix.

The resulting formulations were viscous suspensions. The K-Y matrix appeared to dissolve the PPZHCl, producing a formulation that was a dark orange-brown transparent gel.

**2.2 Standard preparation**

A 10-mg sample of PPZHCl reference material was accurately weighed and placed in a 10-ml volumetric flask. This material was dissolved in and diluted to volume with water, then mixed. The PPZHCl concentration of this stock solution was 1000 µg ml<sup>-1</sup>. A 200 µg ml<sup>-1</sup> PPZHCl working standard was prepared by transferring a 2.00 ml aliquot from the stock solution to a 10-ml volumetric flask,

diluting to volume with the appropriate diluent, then mixing.

### 2.3 Reagents

The following chemicals were used with the assay methods presented: acetonitrile, HPLC grade (Burdick and Jackson); deionized water, Milli-Q System (Millipore); hexane, Reagent grade (Fisher Scientific); hydrochloric acid (concentrated), ACS Certified Plus (Fisher Scientific); acetic acid, HPLC grade (Fisher Scientific); 0.2 M heptanesulfonic acid pre-mixed solution (Alltech Associates, Inc).

A 0.05 M aqueous hydrochloric acid solution was prepared by diluting 4 ml of hydrochloric acid (concentrated) to 1000 ml with deionized water. An ion pairing solution was prepared by combining 25 ml of the heptanesulfonic acid pre-mix with 1000 ml of deionized water to produce a 5 mM heptanesulfonic acid solution. A diluent was prepared by combining 350 ml of acetonitrile with 150 ml of the ion pairing solution.

### 2.4 HPLC instrument parameters

Sample analysis was performed using a Hewlett-Packard liquid chromatograph (Model 1090M, Palo Alto, CA) equipped with a diode-array ultraviolet (UV) detector. Injection volumes of 1  $\mu$ l were eluted through a small-bore C<sub>18</sub> column (ODS-H, 15 cm  $\times$  2.0 mm ID, 5  $\mu$ m particle size, Keystone, Bellefonte, PA) heated to 40 °C. Acetonitrile + ion pairing solution (85 + 15 by volume) mobile phase was used at a flow rate of 0.30 ml min<sup>-1</sup>. Component mixing of the mobile phase was done by the liquid chromatograph. Absorbance data were collected at a wavelength of 280 nm. After each set of analyses, the HPLC column was washed using a set of mobile phase gradients.<sup>9</sup>

### 2.5 Assay of PPZHCl formulations

Each TTD was stripped of the black rubber coating and cut at the tapered end of the device. The bulb and stem pieces were placed in a 50-ml tube and hydrochloric acid (0.05 M; 15 ml) was added to the tube. After 15 min on a mechanical shaker the extract was decanted into a 50-ml volumetric flask. This was repeated with further hydrochloric acid (0.05 M; 2  $\times$  15 ml). The extracts were combined in the 50-ml volumetric flask, and diluted to volume with 0.05 M hydrochloric acid and mixed. A 0.250 ml aliquot of this solution was diluted to 10.0 ml with acetonitrile + ion pairing solution (85 + 15 by volume) for routine analysis. For analysis by HPLC/MS the 0.250 ml aliquot was diluted to 10.00 ml with methanol + water (60 + 40 by volume) containing 1% acetic acid. These solutions were then transferred to a vial and capped prior to analysis.

### 2.6 HPLC/MS instrument parameters

The sample components were analyzed by HPLC/mass spectroscopy (HPLC/MS) using a Model 1100 Hewlett-Packard liquid chromatograph and a LCQ

ion trap mass spectrometer equipped with an electrospray interface (Finnigan-MAT Corp, San Jose, CA). Volumes of 1  $\mu$ l were injected onto a small bore C<sub>18</sub> column (ODS-H, 15 cm  $\times$  2.0 mm ID, 5  $\mu$ m particle size, Alltech, Deerfield, IL) heated to (ambient) 40 °C. The mobile phase was methanol + water (60 + 40 by volume) containing 1% acetic acid at a flow rate of 0.30 ml min<sup>-1</sup>. Component mixing of the mobile phase was done by the liquid chromatograph. Absorbance data were collected at a wavelength of 280 nm. The electrospray needle was held at a potential of +4.5 kV and the capillary inlet was heated to 250 °C. Following tuning and mass calibration, optimal collision-activated dissociation conditions for fragmentation of PPZ was completed by direct infusion of a 100 mg liter<sup>-1</sup> standard solution of PPZ in the mobile phase. The analysis was completed with helium as collision gas at a pressure of 2.0–2.2 mTorr. Collision energy was a percentage of 5.0 V applied to the end caps of the ion trap, typically 20–30% which is 1–1.5 V. The direct infusion line was removed and replaced in-line with the HPLC. Data were collected in the full-scan mode. Full spectra were acquired over  $m/z$  50–400 at 0.5 s scan<sup>-1</sup>.

## 3 RESULTS AND DISCUSSION

### 3.1 Observation of degradate

The PPZHCl/K-Y Jelly formulation was chosen for its homogeneity and ease of handling compared with the other formulations tested. This formulation demonstrated efficacy comparable with Vaseline and corn syrup formulations. An analytical method to evaluate the PPZHCl contents in these TTD formulations was developed using an HPLC assay method.<sup>9</sup> A new TTD known as the 'pipette' device with PPZHCl/K-Y Jelly formulation was designed and a modified PPZHCl assay method was developed. During this work, a previously unreported chromatographic response was observed at a shorter retention time than PPZ (Fig 1). At this point, quality control formulations assayed were at most one month old. Due to sample backlog and workload, the 'pipette' devices prepared by Wildlife Services—Pocatello Supply Depot remained in storage at ambient temperature for approximately a month. Some older devices from previous studies containing the PPZHCl/K-Y Jelly formulation were obtained and this same peak, but with greater magnitude, was observed. Storage under ambient temperatures (20–25 °C) demonstrated a 10% decrease in the active ingredient after 3 months and nearly a 20% decrease after 5.5 months. Interested parties did not want to do a complete reformulation with a new carrier, as K-Y Jelly had attractive handling and formulating characteristics compared with many previously tested carriers such as Karo dark corn syrup, Vaseline, mineral oil and corn oil. We informally checked formulations with glycerine, propylene glycol, alginate in water and

chitinase in water. None proved to have the necessary viscosity nor did they dissolve PPZHCl.

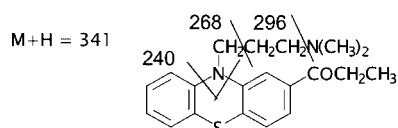
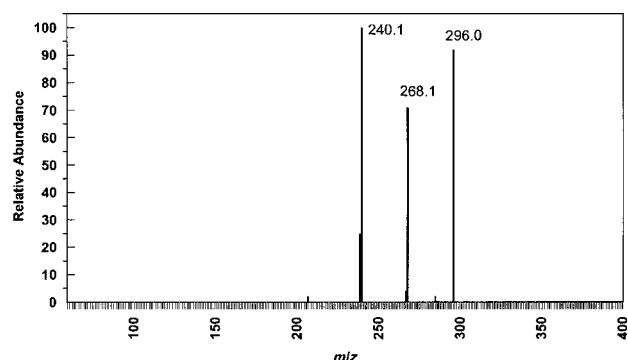
The previously unreported degradate could not be identified solely by UV-visible spectroscopy and therefore a chromatographic system compatible with HPLC/electrospray mass spectrometry (MS) was developed. A column similar to the small bore used for the original formulation was used but instead of the heptanesulfonic acid ion-pairing acetonitrile + water mobile phase an acetic acid (1%) methanol + water system was selected. This was more compatible with the electrospray MS system. An interesting result of this selection was that the degradate component was resolved into three components initially (Fig 2A). With samples of greater age five degradates were observed (Fig 2B).

### 3.2 Identification of degradates

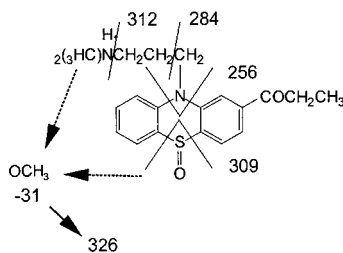
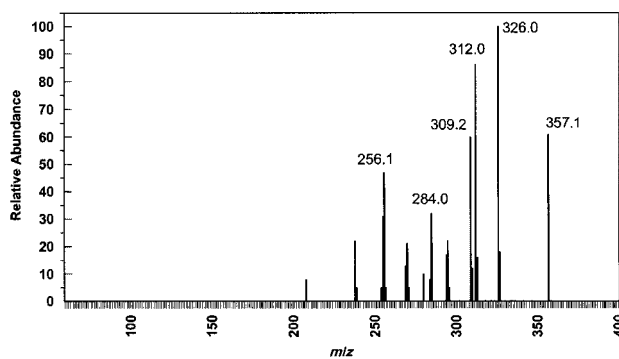
Table 1 lists the molecular ions observed for degradates of PPZ identified. Each molecular ion was collisionally dissociated in the ion trap and the MS/MS mass spectra were recorded. The MS/MS mass spectra of the molecular ions were used to elucidate the structure of the observed chromatographic responses of the PPZ parent molecule and degradates (Figs 3 to 8). The parent molecule PPZ has a molecular ion ( $M + H^+$ ) of 341. Degradate components **A** and **B** in Fig 2A both with a molecular ion of 357 which was an increase in molecular weight of 16 and indicate

**Table 1.** Molecular ions and retention time of the parent molecule (PPZ) and observed degradates

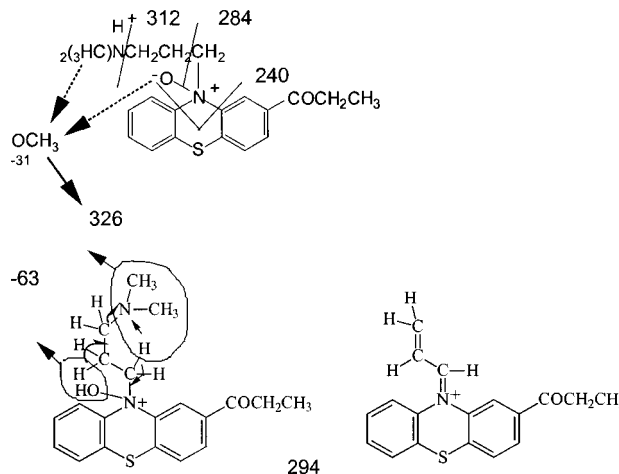
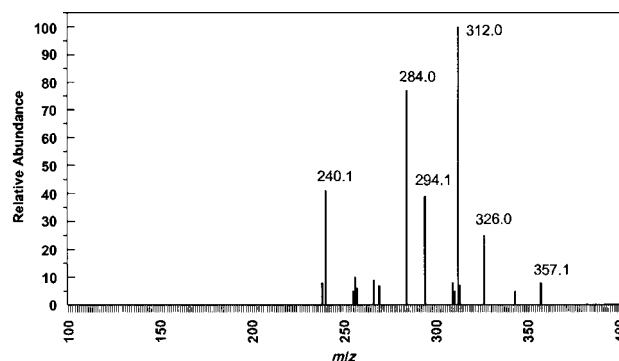
| Peak identification | Retention time (min) | (M + H) <sup>+</sup> m/z |
|---------------------|----------------------|--------------------------|
| PPZH <sup>+</sup>   | 9.00                 | 341.2                    |
| <b>A</b>            | 3.60                 | 357.2                    |
| <b>B</b>            | 4.60                 | 357.2                    |
| <b>C</b>            | 2.45                 | 373.2                    |
| <b>D</b>            | 2.65                 | 345.2                    |
| <b>E</b>            | 5.80                 | 329.2                    |



**Figure 3.** MS/MS mass spectrum of parent molecule PPZH<sup>+</sup> detected at 9.00 min with a molecular ion of 341 and the ion trap scanned over m/z 85.00–360.00.

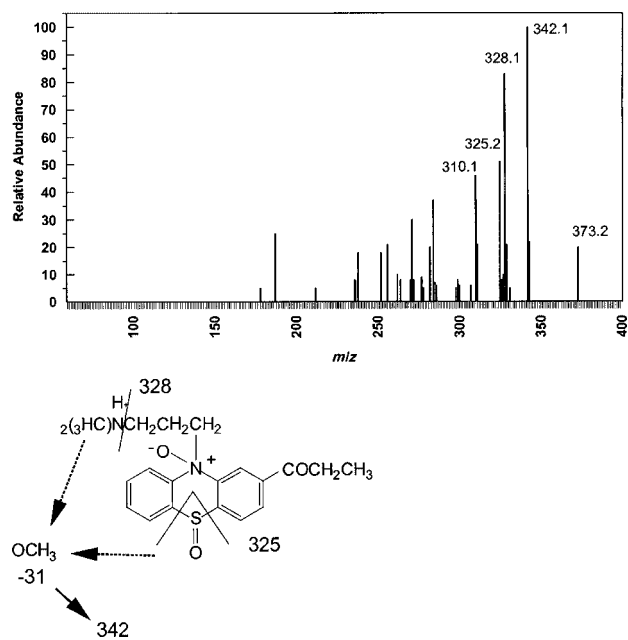


**Figure 4.** MS/MS mass spectrum of degradate **A** detected at 3.60 min with a molecular ion of 357 and the ion trap scanned over m/z 85.00–370.00.

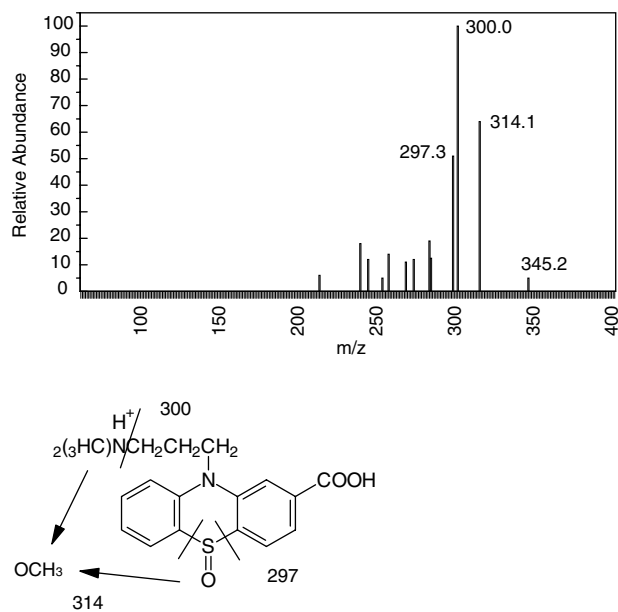


**Figure 5.** MS/MS mass spectrum of degradate **B** detected at 4.60 min with a molecular ion of 357 and the ion trap scanned over m/z 85.00–370.00.

the possible oxidation of the parent molecule. Additional degradates were observed in TTDs stored for 7 months under field conditions from Guam (Fig 2B), Idaho, Minnesota and Utah. As shown in Fig 2B,



**Figure 6.** MS/MS mass spectrum of degradate **C** detected at 2.45 min with a molecular ion of 373 and the ion trap scanned over  $m/z$  90.00–385.00.

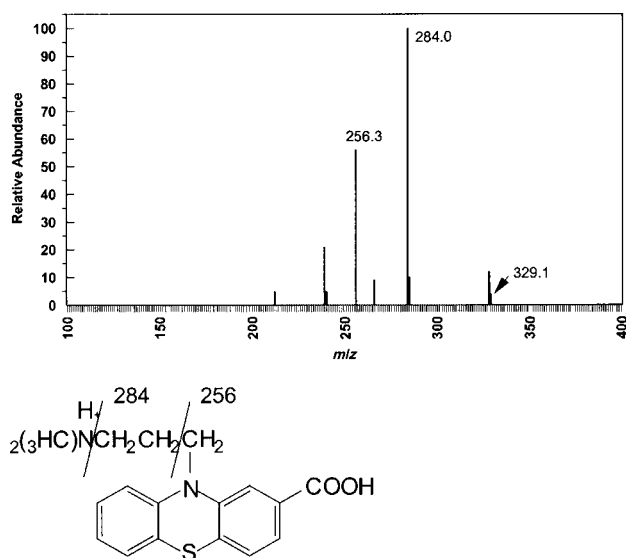


**Figure 7.** MS/MS mass spectrum of degradate **D** detected at 2.65 min with a molecular ion of 345 and the ion trap scanned over  $m/z$  85.00–360.00.

the  $PPZH^+$  peak and the degradate peaks identified by each letter have the molecular ion shown in Table 1.

The MS/MS mass spectrum of the parent molecule  $PPZH^+$  is shown in Fig 3. All of the major fragments ( $m/z$  296, 268 and 240) shown in the mass spectrum occur along the dimethylaminopropyl chain of the molecule.

Degradates **A** and **B** have a molecular ion of 357, which indicates that the parent molecule is singly oxidized, but at two different molecular locations, as indicated by the variation in the MS/MS mass spectra



**Figure 8.** MS/MS mass spectrum of degradate **E** detected at 5.80 min with a molecular ion of 329 and the ion trap scanned over  $m/z$  85.00–360.00.

(Figs 4 and 5). The **A** degradate mass spectrum shown in Fig 4 contains five major fragments ( $m/z = 326, 309, 312, 284$  and  $256$ ) with 326 as the base peak. The **B** degradate mass spectrum also contains five major fragments ( $m/z = 326, 312, 294, 284$  and  $240$ ) with 312 as the base peak as shown in Fig 5. The common collisional dissociation observed between the mass spectra for these two degradates were the mass loss of 31, 45 and 73 mass units. The loss of the 45 and 73 mass fragments observed in the parent  $PPZH^+$  molecule MS/MS mass spectrum indicates fragmentation along the dimethylaminopropyl chain, as does the loss of 101 mass units for degradate **B** and the parent molecule. The common fragment in both **A** and **B** not observed for the parent molecule at  $m/z$  326 represents the loss of 31 mass units and was believed to represent the loss of a methyl group with oxygen as a result of a rearrangement. For **A**, the  $m/z$  of 309 represents the loss of 48 which can be accounted for by the loss of oxygen bonded to the aromatic sulfur. The  $m/z$  fragment at 240 in the MS/MS spectrum of **B** was the loss of 117 mass units that was composed of the amine oxide and the dimethylaminopropyl chain. Degradate **B** is believed to be an amine oxide, as tertiary amines are often oxidized to amine oxides. The  $m/z$  fragment at 294 represents a concerted elimination where dehydration occurs in concert with dissociation of the protonated dimethylamine creating the loss of the 63 fragment (Fig 5).

The MS/MS mass spectra for the minor degradate peaks designated as **C**, **D** and **E** are shown in Figs 6, 7 and 8. The peaks observed at 2.45 and 2.65 min appear to be doubly oxidized. This explains their short retention time, as they are more polar than any of the other degradates. The degradate designated as **C** has  $M + H^+$  373 and the MS/MS mass spectrum indicates that oxygen is bonded to the sulfur, as the loss of 48 is

observed with the  $m/z$  of 325. The other oxygen was most likely bonded to the aromatic amine as indicated by the loss of 63 mass units by the same rearrangement as shown in Fig 5. This creates a rearrangement where dehydration occurs in association with dissociation of the protonated dimethylamine, leaving the  $m/z$  310 fragment which also occurs for degradate **B**.

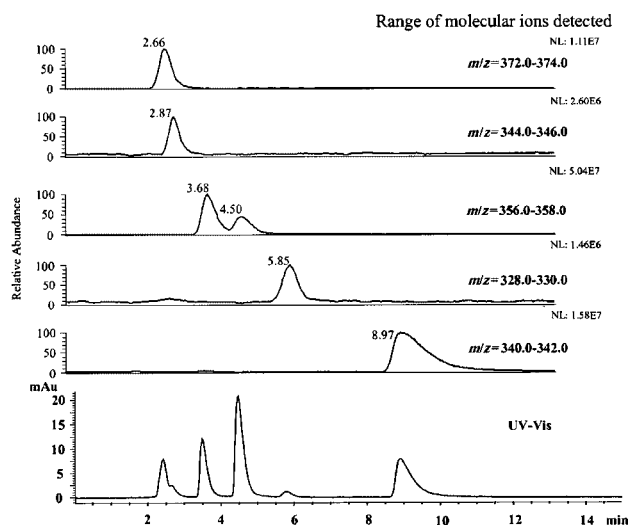
The smaller doubly oxidized degradate (**D**) has a molecular ion of 345. Oxidation of the aromatic sulfur was indicated by the loss of 48 ( $m/z$  of 297). The molecular mass indicates that the propanone group has been oxidized to a carboxylic acid.

The final degradate (**E**) at 5.80 min has  $M + H^+$  329 combined with the presence of fragments at  $m/z$  of 284 (loss of 45 mass units) and 256 (loss of 73 mass units) and the lack of a loss of 101 mass units ( $m/z = 228$ ) from the fragmentation of dimethylaminopropyl plus the aromatic amine indicate that the propanone group has been oxidized to a carboxylic acid.

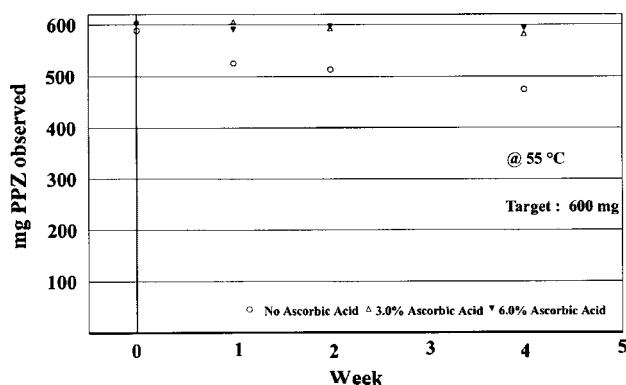
This HPLC/MS chromatographic system can be used to monitor the degree of oxidation for TTD formulations. The molecular ion for the parent molecule and any degradate can be detected and a set of chromatograms can be generated as shown in Fig 9.

### 3.3 Stabilizing the formulation and preventing degradation

The TTD formulation was reformulated to include an antioxidant which was tested for storage stability. TTDs were prepared with 0, 3 and 6% of the antioxidant, ascorbic acid and an additional set of TTDs prepared without PPZHCl as a control blank. The devices were placed in a warm water bath at 55 °C and were assayed at 0, 1, 2 and 4 weeks with the revised formulation method to assess the effectiveness of the antioxidant. These simulated environmental stability studies indicated that the addition of the antioxidant minimized the degradation of PPZHCl (Fig 10).



**Figure 9.** The molecular ion for the parent molecule and any degradate can be detected and a set of chromatograms can be generated for any TTD formulation analyzed by HPLC/MS.



**Figure 10.** Loss of PPZHCl in formulated TTDs with and without the antioxidant ascorbic acid over a four-week period at 55 °C.

## 4 CONCLUSION

During analyses of aged quality-control samples, previously unreported degradates were observed at shorter retention times than the parent compound. Further investigation of formulations stored in trap tap devices at temperatures ranging from 5 to 40 °C during field trials at four different locations confirmed the degradation of the active ingredient. A new analytical method to evaluate propionylpromazine hydrochloride (PPZHCl) degradates in a tranquilizer formulation was developed using HPLC/MS. Further investigation using HPLC/MS/MS identified the degradates as oxidation products of the active ingredient, PPZHCl. PPZHCl reformulated with the anti-oxidant ascorbic acid was utilized to reduce the degradation observed and achieve the required PPZHCl storage stability.

## ACKNOWLEDGEMENTS

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