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# Stability and characterization of perphenazine aerosols generated using the capillary aerosol generator

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**Abstract**

Perphenazine (a potent antiemetic) was aerosolized using capillary aerosol generator to generate respirable condensation aerosols from drug in propylene glycol (PG) solutions, by pumping the liquids through a heated capillary tube. The study characterized the stability of perphenazine during and following aerosol generation. The stability-indicating HPLC method (C-8 column with a mobile phase of 52% 0.01 M pH 3.0 acetate buffer + 48% acetonitrile) also enabled the study of perphenazine stability in solution under acidic, basic, oxidizing and photolysing conditions. An LC–MS (ESI+) method was used to characterize the degradation products. Perphenazine was found to be stable in acidic and basic conditions, while perphenazine sulfoxide was the major product formed in dilute peroxide solutions. Two photo-degradation products were formed in PG that were tentatively identified by LC–MS; one of these was synthesized and confirmed to be 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol. Both photolysis products showed that aromatic dechlorination had occurred and one appeared to also result from interaction with the solvent. Within an aerosolization energy window of 84–95 J, fine particle aerosols were generated from perphenazine PG formulations with no significant degradation. Small amounts of degradation products were produced in all samples during aerosolization at elevated (non-optimal) energies. These were largely consistent with those seen to result from oxidation and photolysis in solution, showing that oxidation and dehalogenation appeared to be the main degradation pathways followed when the CAG system was overheated.


*Keywords:* Perphenazine; Stability; Dechlorination; Aerosol; Particle size

## 1. Introduction

The capillary aerosol generator (CAG) was invented by Howell and Sweeney (Howell and Sweeney, 1998) and further developed by Hindle et al. (Hindle et al.,

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1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004). The device generates fine particle aerosols by pumping liquid formulations through a heated capillary tube. This tube is heated by controlling the electrical energy, which is used to heat and volatilize the formulation, during its passage through the tube. In its present manifestation, which has been described in detail elsewhere (Gupta et al., 2003; Shen et al., 2004), the heating energy used to vaporize the formulation passing through a stainless steel capillary is precisely controlled by computer maintenance of the capillary's electrical resistance. Direct current is passed through the capillary, at the onset of each experiment, to achieve a preselected target resistance, $R_T$, that is proportional to the mean steady state temperature of the capillary (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004). Thus, as larger values are selected for $R_T$, larger amounts of energy are passed through the capillary. In this manner, the physical characteristics of the aerosols can be changed (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004) and temperatures to which the formulation and vapors are exposed can be increased (Gupta et al., 2003; Shen et al., 2004). As a net result, one of the challenges during product development with this method of aerosol generation is to minimize solute decomposition due to overheating (Hindle et al., 1998; Shen et al., 2004) by selecting conditions that preclude significant degradation. This paper serves to illustrate one aspect of the CAG development process whereby a "window" of acceptable energy levels was shown to exist for a drug that was shown independently to be subject to both oxidation and photolysis. Propylene glycol (PG) is a GRAS excipient and was chosen as an excipient for these studies due to its ability to form condensation aerosols following heating in the CAG (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004). PG is an appropriate excipient for pharmaceutical aerosol formulations and has been used previously in nebulizer formulation.

Perphenazine (Fig. 1a), a phenothiazine derivative with strong antiemetic properties, was chosen as the "model drug" for the present study because oral antiemetic therapy often fails and injections are often deemed to be undesirable. However, a rapid onset of systemic action associated with pulmonary delivery is often considered desirable for this class of compounds (Ward et al., 1997; USPDI, 1998). Per-

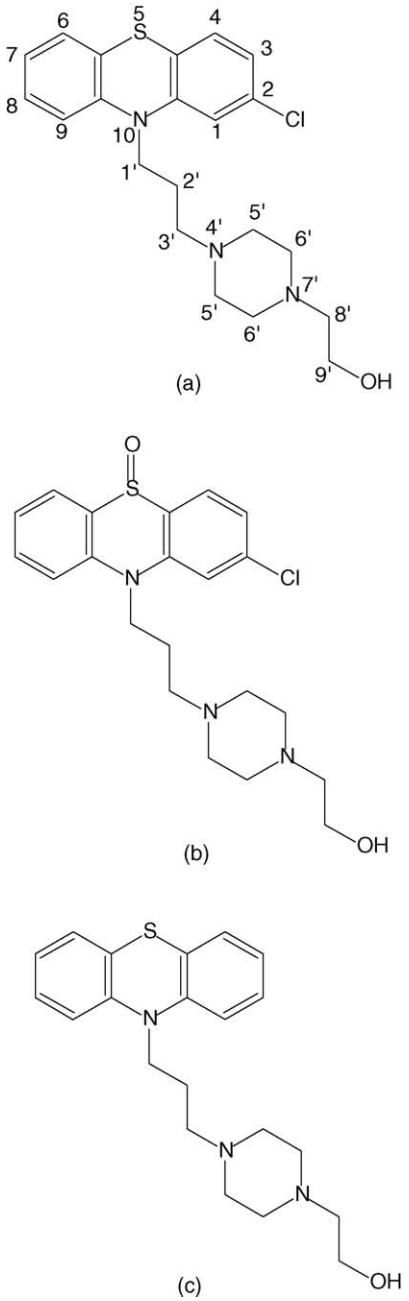


Fig. 1. Structures of (a) perphenazine, (b) perphenazine sulfoxide (compound A), (c) 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol (compound B).

phenazine was also believed to represent a challenge for the CAG because previous studies had shown that similar phenothiazines were easily oxidized and light sensitive (Felmeister and Discher, 1964; Roseboom and Fresen, 1975; Abdel-Moety et al., 1996; El-Gindy et al., 2002). Based on the literature for phenothiazines (Beaulieu and Lovering, 1986; Abdel-Moety et al., 1996; Kumazawa et al., 2000; El-Gindy et al., 2002), HPLC methods had to be developed and validated that were stability-indicating, and that could be used to identify degradation products formed under "high energy" aerosolization conditions. Subsequently, CAG operating conditions could be selected under which perphenazine degradation could be shown to be insignificant.

## 2. Materials and methods

### 2.1. Chemicals

Perphenazine, USP grade, was purchased from Spectrum Chemical Co. (Gardena, CA). Propylene glycol (PG), USP grade, was purchased from Fisher Scientific Co. (Fairlawn, NJ). HPLC grade methanol, acetonitrile and ammonium acetate were purchased from Fisher Scientific Co. USP/FCC grade acetic acid was also purchased from Fisher Scientific Co.; 10 and 30% palladium-carbon was purchased from Sigma–Aldrich Inc. (St. Louis, MO). Silica gel preparative TLC plates were also purchased from Sigma–Aldrich Inc.

### 2.2. Instrumentation

#### 2.2.1. HPLC

The HPLC system consisted of a Waters 2695 separation module and a 2995 photodiode array detector (Waters Corp., Milford, MA). UV absorbance was monitored at 256 nm. The chromatographic separations were performed on a Waters Nova-Pak C-8 column (3.9 mm × 150 mm, 4 μm). The mobile phase was 0.01 M ammonium acetate/acetic acid buffer pH 3.0 and acetonitrile (52:48, v/v). The LC system was operated isocratically at a flow rate of 1.0 mL/min at room temperature. The run time was 16 min in all cases.

#### 2.2.2. LC–MS

The LC–MS analyses were performed on a Waters 2695 separation module as above, with a Waters 996 photodiode array detector online to a Micromass ZMD 4000 single quadrupole mass spectrometer (Waters Corp., Milford, MA). The ESI-MS measurements were carried out in positive ionization mode. For each injection, two scans were performed at cone voltages of 35 and 60 V. Full scan data was obtained over a mass range of *m*/*z* 100–500 and analyzed by Waters Masslynx Software (Version 3.5, Waters Corp., Milford, MA).

### 2.3. Standard solution and HPLC analysis

A stock solution of perphenazine, 268.0 μg/mL, was diluted with mobile phase to make a working solution of 26.8 μg/mL. The working standard was diluted serially to prepare the standard curve, with concentrations ranging 0.01–26.8 μg/mL in mobile phase. Triplicate injections of 60 μL were made and the response ($A_{256nm}$) plotted versus concentration. The LOD and LOQ were determined based upon the RSD of the peak area at the lowest concentrations. The repeatability was tested at a concentration of 5.36 μg/mL over six replicate injections (CDER, 1994). The unknown concentration of the active in the sample was determined using the calibration curve by comparing the peak area of the active with the standard at 256 nm.

### 2.4. Chemical stability of perphenazine

#### 2.4.1. Perphenazine in acidic and basic solutions

Perphenazine was stored in acidic and basic solutions to monitor possible acid or base catalyzed reactions. Due to its poor solubility in water, perphenazine was first dissolved in methanol, then diluted with 0.1N HCl or 0.1N NaOH (nominal perphenazine concentrations were 15.86 ± 0.54 μg/mL, 8% methanol by volume, $n = 3$). The samples were stored at room temperature (24 ± 2 °C) and in the oven (50 ± 2 °C) for 3 days. Aliquots were withdrawn over a period of 3 days, neutralized and assayed using the HPLC method described above.

#### 2.4.2. Perphenazine in $H_2O_2$ solution

Methanolic perphenazine solution was diluted with 0.5% $H_2O_2$ aqueous solution to perform the oxidation study (nominal perphenazine concentration 25.6 μg/mL, 1% methanol by volume) and stored at room temperature (24 ± 2 °C) in the dark. Aliquots

were withdrawn over a period of 5 h and analyzed using the HPLC method above.

*2.4.3. Perphenazine PG solutions in the dark*

Perphenazine solutions in PG (nominal perphenazine concentrations were $4.88 \pm 0.23$ mg/mL, $n = 3$) were stored at room temperature ($24 \pm 2\,^{\circ}C$) for 2 months in the dark to evaluate formulation stability in sealed containers under likely aerosol inhaler reservoir conditions. At the time of assay, approximately 9 mg solution aliquots were accurately weighed, diluted with 5.0 mL mobile phase, and assayed using the HPLC method described above.

*2.4.4. Perphenazine PG solutions under fluorescent light*

At room temperature ($24 \pm 2\,^{\circ}C$), perphenazine in PG solutions (nominal perphenazine concentrations were $4.99 \pm 0.14$ mg/mL, $n = 3$) were exposed to light (Panasonic cool white light, F15T8/CW, Japan; Sylvania cool white light, F15T8/CW, GTE, USA) for 27 h in sealed glass containers. The light strength at the surface of the solutions was estimated to be approximately 3200 lx. Aliquots were weighed accurately at different time points, so that approximately 9 mg of each solution was diluted with 5.0 mL mobile phase, and analyzed by the HPLC method described above.

*2.5. Aerosolization of perphenazine PG solutions*

*2.5.1. Dose capture experiments*

Perphenazine PG solutions of 5.0 mg/mL were aerosolized using a 28 gauge, 44 mm long stainless steel capillary. The pump flow rate was 5.0 μL/s and the run time was 10 s, resulting in a nominal 50 μL delivered volume. Sham experiments ($n = 3$) were performed by collecting the formulation as it was pumped from the capillary tip, in the absence of heating, to determine the actual delivered dose. Different target resistances were employed to provide a range of energies to aerosolize the formulation. Experiments were performed in quintuplicate ($n = 5$) and the total electrical energy consumed was recorded at each target resistance. Dose capture experiments were performed to determine the recovery of the active after aerosolization as described previously (Blondino et al., 2002). Briefly, a glass trap (Research Glass, Richmond, VA) was filled with 10.0 mL collection solvent (HPLC mobile phase in this case), and housed in an ice-water bath to capture the generated aerosol. An air flow of 0.5 L/min was drawn over the capillary tip, to condense the aerosol and bubble it through the collection solvent. The trap was connected to a vacuum line via an additional backup filter (SKC Inc., Eighty Four, PA) in each case. The collection solvents were analyzed by the HPLC method described above.

*2.5.2. Particle size distribution of perphenazine aerosols*

Aerosols were generated under ambient laboratory conditions of a room temperature of $24 \pm 2\,^{\circ}C$ and relative humidities between 20 and 30%. Aerodynamic particle size was determined by the MOUDI$^{TM}$ cascade impactor (MSP Corp., Minneapolis, MN) at a flow rate of $30 \pm 2$ L/min through a USP stainless steel induction port (Marple et al., 1991). After sampling, the impactor was disassembled, the solute and vehicle was collected from the USP induction port and on each MOUDI$^{TM}$ stage impaction surface by washing with a defined volume of solvent. The resulting solutions were analyzed by HPLC. Interstage drug deposition ("wall losses") were pooled and determined by wiping the walls and nozzle arrays of each stage of the impactor with a solvent-moistened tissue and analyzed for perphenazine.

*2.6. Synthesis of the potential degradation product-2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol (Fig. 1)*

Perphenazine (1.6 g) was hydrogenated in acetic acid (50 mL) in the presence of anhydrous sodium acetate (Kishimoto and Uyeo, 1969). In addition, increasing quantities of palladium-carbon were added and the mixture hydrogenated over an 11 day period (additions were made as follows, to the filtrate, after filtration of the mixture: 0.13 g of 10%, 0.13 g of 30%, 0.9 g of 30% and 1.0 g of 10% palladium-carbon at time 0, 72, 120 and 192 h). At 264 h, the mixture was filtered and the filtrate evaporated to dryness under reduced pressure. A solution of 0.1N HCl (50 mL) was added to dissolve the residue and the solution heated under reflux for 12 h. The solution was evaporated to dryness under reduced pressure and about 200 mg of the residue loaded on several silica gel preparative TLC

plates (20 cm × 20 cm, GF 254) and chromatographed using $CH_2Cl_2$:MeOH, 95:5. The fraction with the lowest Rf value (Rf = 0.15) was scraped from the plates, pooled and eluted from the gel using $CH_2Cl_2$:MeOH, 90:10. The eluent was evaporated and 20 mg of the (approximately 50 mg) residue was dissolved in 1.5 mL water with pH adjusted to 12 with concentrated NaOH solution. This solution was then extracted three times into diethyl ether, the ether phase pooled and evaporated under reduced pressure to give approximately 13 mg (0.035 mmol) 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol (Fig. 1). m.p.: 93–95 °C (Literature m.p.: 98–99 °C, GB patent, 1960). $^1$H NMR ($CDCl_3$) $\delta$: 1.96 (m, 2H, H-2′), 2.85 (m, 12H), 3.61 (t, $J$ = 5.4Hz, 2H, H-8′), 3.94 (t, $J$ = 6.9Hz, 2-H, H-9′), 6.92 (m, 4H, aromatic), 7.14, (m, 4H, aromatic). $^{13}$C NMR ($CDCl_3$) $\delta$: 24.0 (C-2′), 44.8 (C-1′), 52.4 (C-5′), 52.9 (C-6′), 55.2 (C-3′), 57.3 (C-8′), 58.7 (C-9′), 115.0 (C-1, C-9), 121.9 (C-4, C-6), 124.6 (C-2, C-8), 126.7 (C-3, C-7), 127.0 (C-4a, C-5a), 144.8 (C-9a, C-10a).

## 3. Results and discussion

### 3.1. Stability-indicating HPLC method

The HPLC assay method for perphenazine was found to be linear in the range 0.03–26.8 μg/mL. The correlation coefficient of the regression line was 0.9999 (nine concentrations measured in triplicate). The limit of detection for perphenazine was 0.01 μg/mL and the limit of quantitation was 0.03 μg/mL (the RSD of peak area at these two concentrations was 6.3 and 3.0%, respectively). The injection repeatability was found to be 0.25% (CDER, 1994). Fig. 2 shows the chromatograms for mixtures of perphenazine and its degradation products (oxidation product A and the photo-degradation products B and C described below). The UV method was clearly stability-indicating with good baseline separation of the active from its major degradation products formed under the stress conditions described below. Oxidation to the sulfoxide was the most effective form of accelerated decomposition (see below) where the apparent concentration of perphenazine was shown to decay to <10% of its starting value in the presence of hydrogen peroxide.

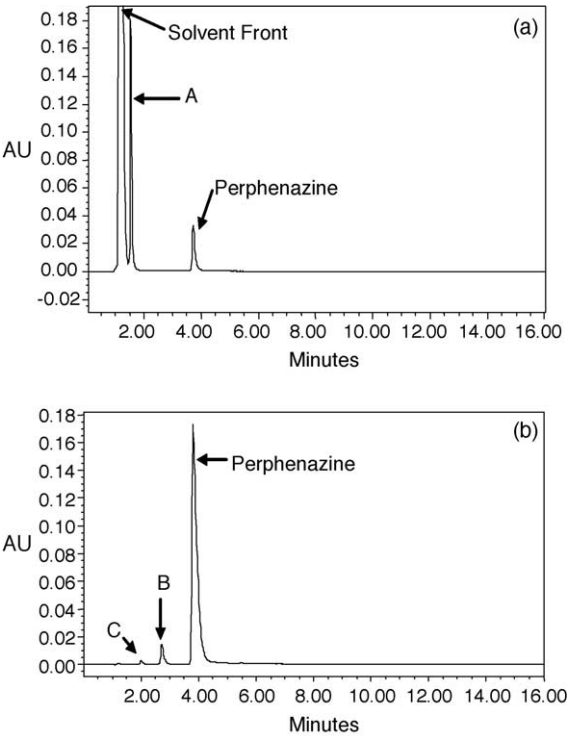


Fig. 2. The UV chromatogram (λ = 256 nm) of perphenazine and its degradation products. (a) Perphenazine and the oxidation product—compound A formed in 0.5% $H_2O_2$; (b) perphenazine and the photo-degradation products—compounds B and C formed in propylene glycol.

### 3.2. Chemical stability of perphenazine under stress conditions

The duration of each stability study was determined by the relative rates of degradation. Following exposure to acidic and basic conditions for 3 days, the apparent concentrations of the active were expressed relative to their values at time zero. The mean recoveries ±S.D. (percentage remaining) in acidic solutions were 100.25 ± 1.79 and 99.06 ± 2.72 at 24 ± 2 and 50 ± 2 °C, respectively. In basic solutions, the corresponding values were 96.75 ± 2.21 and 97.29 ± 1.91 (mean ± S.D.), respectively. The recoveries were within the acceptance criteria of 90–110% for perphenazine according to USP (1999) and no chromophoric degradation products with areas larger than 0.5% of the total were detected.

Table 1
The retention time, UV maximum absorbance, parent ion and fragment ions of the degradation products and standards

| Compound | Retention time (min) | $UV_{max}$ (nm) | Base peak[a] | Fragment ions[b] *m/z* (relative intensities) |
|---|---|---|---|---|
| A | 1.55 | 238, 275, 300, 343 | 420 | 171 (84)<br>143 (75)<br>246 (35) |
| B | 2.7 | 254 | 370 | 171 (100)<br>143 (98)<br>212 (38) |
| C | 2.0 | 254 | 444 | 171 (100)<br>143 (48)<br>286 (10) |
| D | 1.7 | 229 | 145 | 145 (100)<br>162 (3)<br>186 (5) |
| Perphenazine sulfoxide | 1.55 | 238, 276, 300, 343 | 420 | 171 (93)<br>143 (81)<br>246 (25) |
| 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol | 2.7 | 254 | 370 | 171 (100)<br>143 (98)<br>212 (29) |

[a] Data collected at a cone voltage of 35 V.
[b] Data collected at a cone voltage of 60 V.

Perphenazine exposure to hydrogen peroxide produced rapid degradation, in the most part (>90%) to degradation product compound A (Fig. 2a). Compound A was confirmed to be perphenazine sulfoxide (Fig. 1b) by comparing chromatograms and mass spectra with those of (±) perphenazine sulfoxide from USP (Rockville, MD; Table 1). Perphenazine sulfoxide showed an $[M+H]^+$ ion at $m/z = 420$ with cone voltage at 35 V. At a cone voltage of 60 V, predominant fragmentation ions were at 143, 171 and 246, similar to perphenazine, as described below. Perphenazine sulfoxide accounted for 22.0% of the total peak area after 109 min in 0.5% hydrogen peroxide solution. The degradation of perphenazine followed an apparent first-order process ($k = 0.063\ min^{-1}$, Fig. 3a), with kinetics similar to the degradation of phenothiazine and promethazine in oxygen saturated media (Roseboom and Perrin, 1977a; Underberg, 1978). One other minor degradation product (<0.5% of total area at all sample times) was observed but not identified.

### 3.3. Stability of perphenazine in propylene glycol solutions

Perphenazine was stable when formulations suitable for aerosolization in CAG (solutions in PG) were stored in the dark. The apparent concentrations of the active after 60 days dark storage at room temperature were expressed relative to their values at time zero. The mean recovery ±S.D. (percentage remaining) was 102.10 ± 2.84. The recoveries were within the range of 90–110% and no chromophoric degradation products with areas larger than 0.5% of the total were detected.

Similar formulations stored under fluorescent light showed apparent zero-order degradation ($k = 82.34\ \mu g/mL\ h$; Fig. 3b) with kinetics similar to those reported for chlorpromazine hydrochloride under UV irradiation (Felmeister and Discher, 1964). The HPLC method provided baseline separation of the active from its two major degradation products (compounds B and C; Fig. 2b). After 5.5 h of light

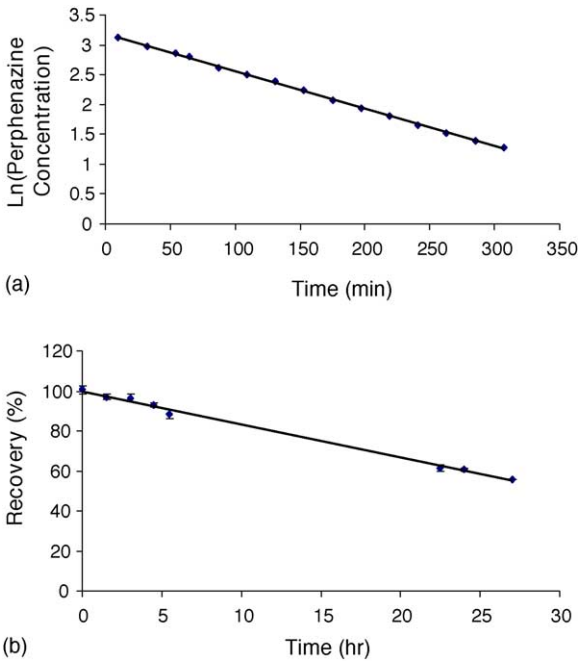


Fig. 3. Perphenazine degradation profiles (a) in 0.5% $H_2O_2$ solution; (b) in PG solution stored under fluorescent light.

exposure, the area percentages of compounds B and C were 6.6 and 1.1%, respectively.

### 3.4. Molecular structures of degradation products

Degradation products were evaluated by LC–MS at two cone voltages and their structures proposed (Fig. 4b and c). Generally speaking, at 35 V, $[M + H]^+$ was the base peak and fragment ions were of low intensities. When the cone voltage was increased to 60 V, fragment ions were produced by collision-induced dissociation; the intensities of the parent ion decreased and fragment ions increased with the exception of compound D, as shown below. Perphenazine showed an $[M + H]^+$ ion of 404 at a cone voltage of 35 V (Fig. 4a). At 60 V, its predominant fragment ions were *m*/*z* 143 and 171, corresponding to the piperazine side chain. Another fragment ion at *m*/*z* 246 showed the chlorine isotope pattern, corresponding to the tricyclic ring. The fragmentation of perphenazine was in agreement with the data in the literature (Kumazawa et al., 2000).

At a cone voltage of 35 V, compound B showed a base peak of 370, which is 34 mass units lower than perphenazine (Fig. 4b). The lack of the chlorine isotope pattern, observed for the base peak ion, indicated the loss of chlorine from the aromatic ring upon photo irradiation. At increased cone voltage, the predominant fragment ions were *m*/*z* 143 and 171, corresponding to the piperazine side chain; and *m*/*z* 212, corresponding to the tricyclic ring. The cleavage of the carbon–chlorine bond has been reported in the literature for chloroaromatic compounds under photolytic conditions (Pinhey and Rigby, 1969). In addition, dehalogenation reaction of phenothiazine derivatives has been induced during the FAB-MS process (Edom et al., 1991). It has also been reported that dechlorinated metabolites appeared in plasma following administration of chlorpromazine (Sgaragli et al., 1986).

In order to confirm the structure of (the dechlorinated) compound B, however, 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol (Fig. 1c) was synthesized. The m.p., $^1$H NMR and $^{13}$C NMR spectra were consistent with the structure proposed (GB patent, 1960; Post et al., 1980; Abadi et al., 1999). Analysis of the product by the HPLC method described previously indicated that it had the same retention time, UV spectrum and MS spectrum as the photodegradation product B (Table 1). Compound B was confirmed to be 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol.

Compound C had a base peak of 444 at cone voltage of 35 V; once more there was no chlorine isotope pattern observed (Fig. 4c). At an increased cone voltage of 60 V, fragment ions of *m*/*z* 143, 171 and 286 were observed. Again, *m*/*z* 143 and 171 corresponded to the liberation of the side chain as seen with perphenazine and compound B. A fragment ion of *m*/*z* 286 may correspond to the tricyclic ring. Compared with compound B, the *m*/*z* of both the parent ion and fragment ion of the tricyclic ring for compound C was 74 mass units higher, that suggested the nucleophilic substitution of propylene glycol to the tricyclic ring during photo irradiation. Although the position of the substitution was not determined, it is probably on the C-2 or C-3 position, as the activation of the site (C-2) meta to the electron withdrawing group on an aromatic system under photo conditions has been reported (Horspool, 1976), and for phenothiazines, C-3 and C-7 have the highest electron density enabling their reaction with a nucleophile (Roseboom and Perrin, 1977b).

### 3.5. Aerosolization of perphenazine PG solutions

#### 3.5.1. Dose capture experiments with CAG

The mass of the active recovered after aerosolization was expressed relative to the mass recovered from sham experiments performed in the absence of heating. Dose capture experiments were performed at a volumetric airflow rate of 0.5 L/min to enable total capture of the aerosol. The formulation mass flow rate and heating profile of the capillary was unaffected by the lower volumetric airflow rate. The dose capture results are shown as percentage recoveries in Table 2 at different target resistances. No statistically-significant difference in recoveries at the 5% confidence level was seen within the energy window of 84.5–95.1 J. The theoretical energy required to vaporize the target mass

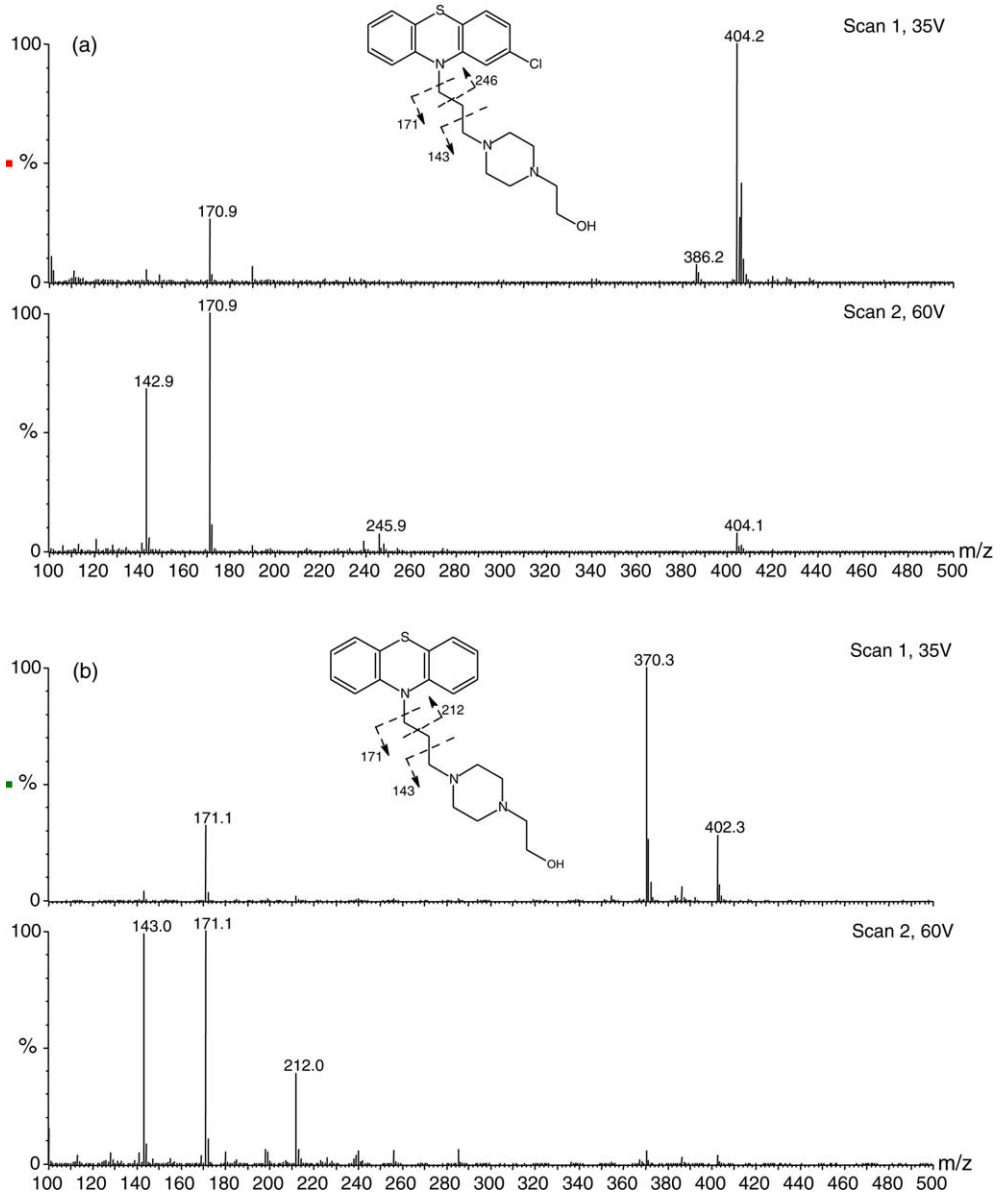


Fig. 4. Mass spectra of perphenazine and its degradation products: (a) perphenazine; (b) compound B; (c) compound C.

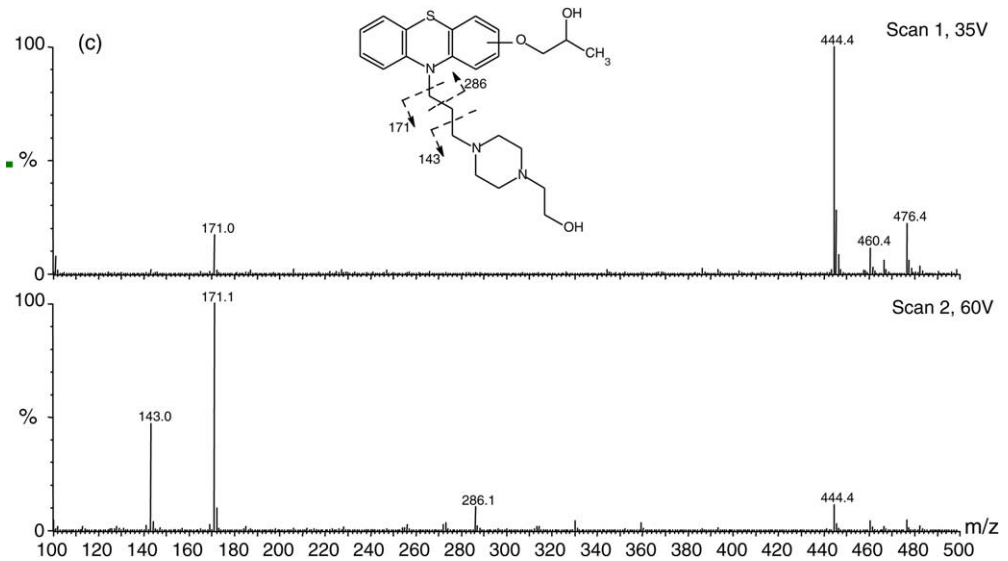


Fig. 4. (*Continued*).

of this formulation according to Shen et al. (2004) was 76.7 J. Values less than the theoretical energy are known to produce poor aerosols in CAG (due to incomplete vaporization) and it was clear that perphenazine degradation began to show at energies higher than 100 J (Table 2). Thus, even for this oxidation-prone molecule, a significant window existed for successful CAG operation and perphenazine aerosol production, within which significant thermally-induced degradation did not occur.

### *3.5.2. Stability of perphenazine in CAG condensation aerosols*

Two degradation peaks (compounds B and D) could be detected in dose capture samples at energies around 84 J while a third degradation peak (compound A) was detected when aerosol samples were generated at energies of approximately 100 J. Compound B was also increased at 100 J compared with samples generated at lower energies. At the highest tested energy level of 130 J, three degradation peaks (compounds A, B and D) could be seen in all five dose capture samples where the mean peak areas for A, B and D accounted for $0.13 \pm 0.02$, $0.28 \pm 0.03$ and $0.15 \pm 0.01$% of the total peak area, respectively. The LC–MS method was employed to evaluate these degradation products, their retention times, UV maxima and mass spectra (Table 1).

Compounds A and B were found to be perphenazine sulfoxide and 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol by comparing with the standards described above. Compound D had a base peak of 145 (35 V cone voltage) and additional peaks of *m*/*z* 162 and 186 may have been due to ammonia and acetonitrile adduct ions, respectively. At a cone voltage of 60 V, the intensities of *m*/*z* 162 and 186 decreased, while *m*/*z* of 145 remained the most abundant. The chlorine isotope pattern was observed for each of the three major peaks and this compound was not identified based on the LC–MS results. Overall in this study, minor degradation products could be detected in the aerosols produced by this CAG, provided the equip-

Table 2
Results of dose capture experiments (mean ± S.D.; $n = 5$)

| Perphenazine concentration (mg/mL) | Energy (J) | Recovery (%) |
|---|---|---|
| 4.96 | 84.45 ± 0.80 | 100.81 ± 1.46 |
| 4.96 | 89.97 ± 0.75 | 100.84 ± 2.47 |
| 4.98 | 95.09 ± 0.51 | 98.89 ± 0.86 |
| 4.98 | 100.24 ± 0.75 | 95.27 ± 2.04 |
| 4.98 | 115.04 ± 1.58 | 92.57 ± 2.15 |
| 5.05 | 130.59 ± 1.55 | 93.53 ± 0.91 |

Table 3
Results of MOUDI cascade impaction experiments (mean ± S.D.; $n = 5$)

| Perphenazine concentration (mg/mL) | Energy (J) | Recovery (%) | MMAD (μm) | $Dv_{10}$ (μm) | $Dv_{90}$ (μm) | Induction port deposition (%) | Fine particle fractions (%) | Wall losses (%) |
|---|---|---|---|---|---|---|---|---|
| 4.97 ± 0.01 | 85.12 ± 1.62 | 97.87 ± 2.75 | 0.39 ± 0.03 | 0.06 ± 0.01 | 0.98 ± 0.05 | 3.0 ± 1.0 | 94.41 ± 1.00 | 1.7 ± 0.7 |
| 4.98 ± 0.01 | 90.39 ± 0.85 | 97.51 ± 3.18 | 0.42 ± 0.02 | 0.06 ± 0.00 | 1.02 ± 0.09 | 2.9 ± 0.8 | 94.53 ± 1.30 | 1.9 ± 0.6 |
| 4.97 ± 0.01 | 96.18 ± 0.76 | 96.13 ± 2.87 | 0.39 ± 0.04 | 0.06 ± 0.01 | 0.98 ± 0.07 | 2.2 ± 0.9 | 94.12 ± 1.56 | 2.3 ± 1.3 |

ment was operated at or above the upper end of the acceptable energy window (Table 2). Oxidation and dehalogenation appeared to be the main degradation pathways under these conditions.

### 3.5.3. *Particle size distribution of perphenazine aerosols*

Previous studies have shown that the particle size distribution of CAG aerosols was not affected by variability of the ambient RH conditions. Hong et al. (2002) reported no significant change of benzyl MMAD following generation of aerosols in relative humidities between 24 and 70% RH. Therefore, these studies were similarly performed under ambient laboratory conditions. MOUDI$^{TM}$ cascade impaction experiments were performed within the optimal energy window described previously. Mean recoveries from the cascade impactor apparatus were >96% (Table 3). Hundred percent recovery of the active is not expected due to the nature of these experiments (Shen et al., 2004). The perphenazine mass median aerosol diameters (of the drug mass collected in the impactor) were <1 μm and consistent with previously reported results for these collection conditions (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003). Fig. 5 shows the mean particle size distribution of a perphenazine aerosol generated at 90.39 ± 0.85 J ($n = 5$). There was significant fraction of ultrafine drug particles on the filter and lower stages of MOUDI$^{TM}$ cascade impactor. Previous studies have shown that condensation aerosol formation takes place within 1 cm of the capillary tip (Shen et al., 2004). This suggests that the significant submicron perphenazine aerosol fraction is due to aerosol droplets rather than perphenazine vapor. MOUDI wall losses were small and less than 2.5% of total recovery on average. Values for fine particle fraction (FPF) in Table 3 are the percent of the total mass recovered with aerodynamic diameters <5.6 μm.

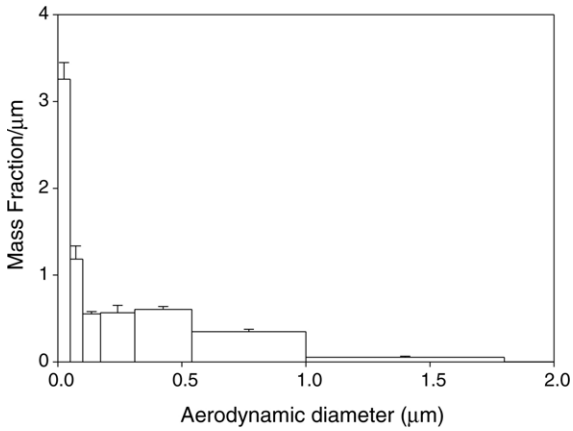


Fig. 5. Aerodynamic particle size distribution of perphenazine aerosols generated by the CAG from 4.98 mg/mL perphenazine PG solution at 90.39 ± 0.85 J ($n = 5$) at formulation flow rate of 5.0 μL/s.

## 4. Conclusions

A stability-indicating HPLC method for the analysis of perphenazine was developed. The method was capable of resolving perphenazine from its major degradation products formed under a wide variety of conditions. Drug in PG solutions were stable when stored at room temperature in the dark. A large energy window existed for successful CAG condensation aerosol production from these solutions, within which potentially highly respirable, sub-micrometer aerosol production was possible, in the absence of statistically-significant degradation. Breath-hold and hygroscopic growth of PG aerosols may improve the in vivo lung deposition of these submicron aerosols. In solution, perphenazine sulfoxide was the major oxidation product formed in dilute hydrogen peroxide and two further degradation products were formed when dissolved perphenazine was exposed to light in propylene gly-

col. These degradants were tentatively identified using LC–MS and one of their structures was confirmed by synthesis. Both showed an apparent absence of perphenazine's aromatic chlorine and one appeared to be the product of a reaction between the drug and PG. When the capillary aerosol generator was operated at the upper end of its acceptable energy window (to evaporate and form condensation aerosols of perphenazine in PG), minor degradation products of this labile compound could be detected in collected aerosols. These were largely consistent with those seen to result from oxidation and photolysis in solution, showing that oxidation and dehalogenation appeared to be the main degradation pathways followed when the CAG system was overheated.

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