COGENT
Phenyl Hydride™

Promethazine Assay & Impurity Methods

Extended Application Note

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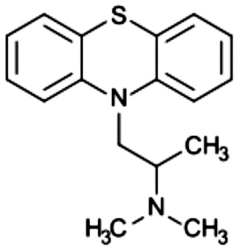
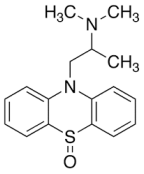
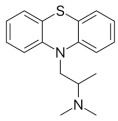


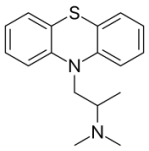
Promethazine



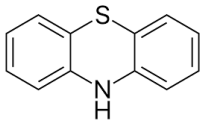
Promethazine sulfoxide



N-Desmethyl promethazine derivative



Iso-Promethazine



Phenothiazine

Assay Method:

70% A: DI H_2O + 0.1% TFA
30% B: Acetonitrile + 0.1% TFA

Flow rate: 1.5 mL/min
Detection: 254 nm
Injection Volume: 5.0µL
Temperature: 25°C

Impurities Method:

A: DI H_2O + 0.1% TFA
B: Acetonitrile + 0.1% TFA

Time (min.)	%B
0	14
1	14
45	28
64	80
65	14

Flow rate: 2.0 mL/min
Detection: 0–48 min 254 nm; 48–65 min 320 nm
Injection Volume: 15.0µL
Temperature: 30°C

Introduction

Promethazine is a phenothiazine-class compound with a variety of pharmaceutical applications. Its primary use is as an antihistamine to relieve symptoms of various allergic reactions. Like other first-generation antihistamines, it has strong sedative properties and can be applicable in treating insomnia. It exhibits anticholinergic properties as well and can reduce nausea and motion sickness. It also helps to reduce symptoms of the common cold such as cough, runny nose, and sneezing.

In routine assay and impurity analyses of promethazine HCl formulations, there are a few potential obstacles to consider. One problem is that promethazine has amine groups which are known to produce tailing with residual silanols that may be present on an HPLC column. This tailing can result in inaccurate quantitation and possible interference with peaks eluting in the tail portion. Another issue is that obtaining adequate selectivity between promethazine and some of its main impurities can be difficult. Iso-promethazine is an isomer and hence may be difficult to distinguish using a reversed phase mechanism alone.

Use of Cogent TYPE-C Silica™ columns was able to address these challenges due to the unique nature of the silica hydride surface and the secondary selectivity characteristics of the bonded ligands. In this study, both assay and impurity methods were developed for promethazine tablets. The method viability was assessed by comparing established system suitability requirements with the data that was obtained. Quantitation of promethazine and its specified impurity, promethazine sulfoxide, was performed.

Experimental

Materials

Trifluoroacetic acid (HPLC-grade ampules), phenothiazine, and promethazine HCl were from Sigma–Aldrich (St. Louis, MO). Iso-promethazine was from LGC (Luckenwalde, Germany). Promethazine sulfoxide and N-desmethyl promethazine derivate were from TRC (Toronto, Canada). Deionized water (DI H_2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

Instrumentation

A Hewlett–Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength UV detector was used. The system was interfaced with Agilent Chemstation™ (Santa Clara, CA, USA) software. For the assay method, a 4.6 x 150 mm Cogent Phenyl Hydride™ column was used, with a particle size of 4µm, and a pore size of 100Å. For the impurity method, a 4.6 x 150 mm Cogent UDC-Cholesterol™ column was used, with a particle size of 4µm, and a pore size of 100Å. Two methods were developed, one for promethazine HCl assay and another for impurities. The conditions used in each method are shown in the tables at the left.

Sample Preparation

ASSAY METHOD

Tablet. Twenty 12.5mg strength promethazine HCl tablets (99.9% purity) were weighed. Masses (g) were as follows:

0.2023	0.1995	0.1995	0.2019
0.2011	0.2001	0.1985	0.2014
0.1992	0.2011	0.2000	0.2008
0.1982	0.2016	0.1995	0.1997
0.1998	0.2002	0.2003	0.2023

Average: 0.2004 g. Sum: 4.0070 g. All twenty whole tablets were added to a 500 mL low-actinic volumetric flask. Next, a premixed diluent of 50/50/0.1 DI water/ acetonitrile/ TFA was prepared. 250 mL of the diluent was added to the flask with the tablets. The flask was sonicated for 10 min and then shaken mechanically by a vortexer for 1 hr. The flask was then swirled by hand and diluted to mark with the diluent. It was capped and inverted several times to ensure thorough mixing. The entire solution was vacuum filtered through a 0.45µm
nylon membrane filter. Using a volumetric pipet, a 20 mL aliquot of the filtrate was transferred to a 200 mL low- actinic volumetric flask. The flask was then diluted to mark with the diluent. Then it was capped and inverted several times. A portion was filtered with a 0.45µm nylon syringe filter. This solution was used for injections.

Reference Standard. On an analytical balance, 25.0 mg promethazine HCl reference standard was weighed on a tared weigh boat. The material was quantitatively transferred to a low-actinic 100 mL volumetric flask. A premixed diluent of 50/50/0.1 DI water/ acetonitrile/ TFA was prepared. 50 mL diluent was added to the flask with the reference standard in it. It was sonicated 10 min, diluted to mark with the diluent, capped, and inverted several times. Using a volumetric pipet, a 10 mL aliquot was transferred to a 50 mL low-actinic volumetric flask. After diluting to mark with the diluent, the flask was capped and inverted several times. Then a portion was filtered (0.45µm, nylon) with a syringe filter and used for injections.

IMPURITY METHOD

Tablet. Twenty 12.5mg strength promethazine HCl tablets (99.9% purity) were weighed. Masses (g) were as follows:

0.1974	0.1995	0.1995	0.2019
0.2030	0.2001	0.1985	0.2014
0.1991	0.2011	0.2000	0.2008
0.2025	0.2016	0.1995	0.1997
0.2002	0.2002	0.2003	0.2023

Average: 0.2004 g. Ground all twenty tablets and added 400 mg to a 25 mL low-actinic volumetric flask. Next, a premixed diluent of 50/50/0.1 DI water/ acetonitrile/ TFA was prepared. 12.5 mL of the diluent was added to the flask with the ground tablet material. The flask was sonicated for 10 min. The flask was then swirled by hand and diluted to mark with the diluent. It was capped and inverted several times to ensure thorough mixing. A portion was filtered with a 0.45µm nylon syringe filter and used for injections.

Reference Standards. Prepared individual solutions of 10 ppm phenothiazine, 100 ppm N-desmethyl promethazine derivate, 20 ppm promethazine sulfoxide, and 10 ppm iso- promethazine with a diluent of 50/50/0.1 DI water/ acetonitrile/ TFA. For promethazine reference standard solution, 25.0 mg promethazine HCl was transferred to a 25mL low-actinic volumetric flask. 12.5 mL of a 50/50/0.1 DI water/ acetonitrile/ TFA diluent was added and the flask was sonicated 10 min. Then it was diluted to mark, capped, and inverted several times. Using an automatic delivery pipet, a 2 mL aliquot was transferred to a 200 mL low-actinic volumetric flask. It was diluted to mark with the diluent, capped, and inverted several times. A portion was filtered with a 0.45µm nylon syringe filter and used for injections.

Results and Discussion

ASSAY METHOD

Using the Phenyl Hydride™ column, a highly symmetrical peak shape was obtained for promethazine in a tablet extract matrix (**Fig. 1**). The mobile phase is a simple isocratic solvent system and can be quickly prepared by the QC laboratory technician. The following formula was used to calculate % promethazine HCl:

% promethazine HCl = [Spl_{area} / Std_{area}] x [Ave_{wt}/Spl_{wt}] x F

F= [(Std_{wt}/100 mL) x (Dil) x (pur)] / [LC/900 mL]

where Spl_{area} is the sample area response (mAU x min), Std_{area} is the average area response of the standard (mAU x min), Ave_{wt} is the average weight of the tablets (g), Splwt is the total weight of the tablets (g), Std_{wt} is the exact weight of promethazine HCl standard (mg), LC is the active label claim (mg), pur is the standard purity percent factor (%), Dil is the dilution, and F is a calculation factor. The values obtained in the data are shown in Table I.

The value for % promethazine HCl using the formula and the data was calculated to be 102.5%, which is within the acceptance criterion of 95–110%.

System suitability of the method was evaluated according to the following acceptance criteria:

- %RSD of the promethazine peak area must be not more than 2.0%
- Tailing factor must be not more than 2.0
- Plate count must be not less than 1000

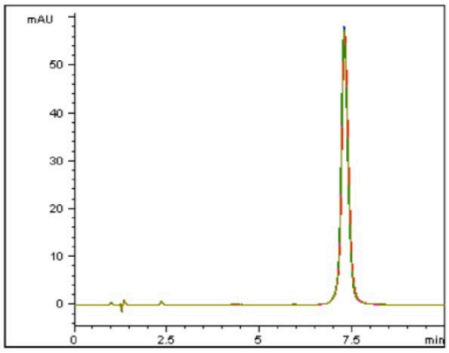


Figure 1

Overlay of 5 runs

Table I

name	value
Spl_{area}	773.11 mAU*min
Std_{area}	753.23 mAU*min
Std_{wt}	25.0 mg
Spl_{wt}	4.0070 g
Ave_{wt}	0.2004 g
LC	12.5 mg
Pur	99.9%
Dil	20
F	1998

Table II

Injection #	N	T_f	Area
1	8055	1.089	777.366
2	7799	1.093	777.452
3	8044	1.084	770.902
4	7776	1.078	771.479
5	7975	1.085	768.330
average	7930	1.086	773.106

The values obtained in each injection for efficiency (N), tailing factor (Tf), and area are given in Table II. The %RSD for area was calculated to be 0.53%, which is within the specification of no more than 2.0%. Likewise, the values for N and Tf were all within the system suitability limits.

IMPURITY METHOD

The development of a suitable method for separation and detection of the main impurities in the promethazine tablet formulation had several notable challenges. Using reference standards and the Phenyl Hydride™ column, the iso-promethazine peak was observed to co-elute with promethazine and no selectivity could be obtained among the two peaks using any studied conditions. However, separation was observed using the UDC-Cholesterol™ column instead. This column can provide shape selectivity as well as reversed phase interactions, and this may account for the difference in separation compared to the Phenyl Hydride™ stationary phase. Due to the differences in hydrophobicity among the studied impurities, a gradient was required instead of the simpler isocratic mobile phase used in the assay method. Even so, a relatively long and shallow gradient was needed so that the critical peak pair, promethazine and iso- promethazine, could separate adequately (**Fig. 2**). Peak identities were assigned using individual standards.

1. Promethazine sulfoxide
2. N-Desmethyl promethazine derivate
3. Promethazine
4. Iso-promethazine
5. Phenothiazine

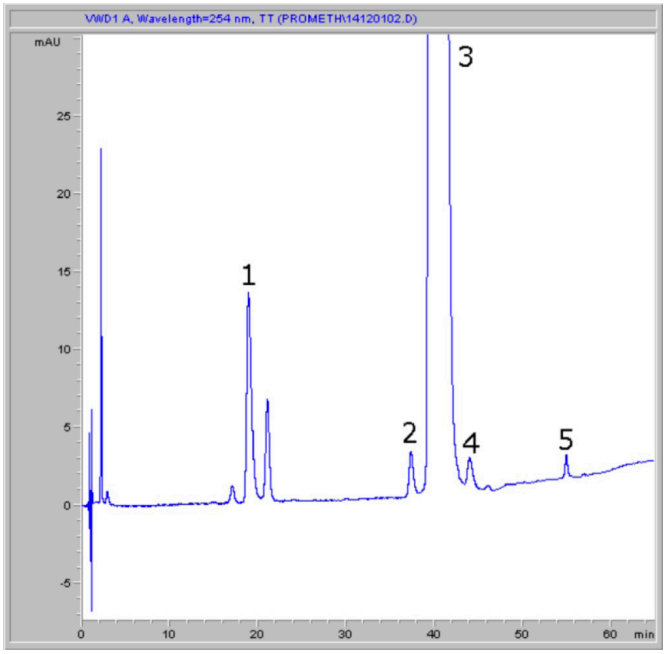


Figure 2

Another problematic issue was that, as the gradient progressed, the baseline signal began to drastically slope upwards, making detection of phenothiazine (peak 5) difficult. Fortunately, phenothiazine has a UV max at 320 nm, and interference from the background is low in this region. Therefore, a wavelength change was used after iso- promethazine eluted. With a 15µL injection volume, the signal for peak 5 was strong enough to be observed. In order to minimize background noise, it is advisable to use high- purity, single-use TFA ampules.

Once an acceptable method was devised, quantitative impurity calculations could be performed. The formula for determining % impurity is as follows:

$$\% \text{ impurity} = [Spl_{area}/Std_{area}] \times [Ave_{wt}/Spl_{wt}] \times F$$

$$F = [(Std_{wt}/25 \text{ mL}) \times (2.0 \text{ mL}/200 \text{ mL}) \times (pur)] / [LC/25 \text{ mL}]$$

Values for each parameter are shown in Table III (Spl_{area} corresponds to the peak area of the specified degradation product promethazine sulfoxide). The value calculated for % impurity of promethazine sulfoxide using these values and the equations was 0.17%. The limit for this impurity is specified to be not more than 0.20%, and therefore this sample is within the limit.

Table III

name	value
Spl_{area}	26.0281 mAU*min
Std_{area}	151.093 mAU*min
Std_{wt}	25.0 mg
Spl_{wt}	0.4000 g
Ave_{wt}	0.2004 g
LC	12.5 mg
Pur	99.9%
F	1.998

Conclusion

The Phenyl Hydride™ column produced excellent peak shape for this tertiary amine-containing compound in the QC assay method. Quantitative calculations for percent promethazine were within the acceptable 95–110% range, and the %RSD, plate count, and tailing factor met the system suitability criteria. The UDC-Cholesterol™ column was found to be suitable for impurity analysis of the promethazine tablets since it was able to resolve the critical pair of promethazine and iso-promethazine. All the impurities were well-resolved using a gradient approach. Switching to a higher wavelength near the end of the gradient allowed for the detection of the last eluting impurity with minimized interference from the baseline. Quantitation of the specified degradation product promethazine sulfoxide was possible using the method, and its level was found to be under the specified acceptable upper limit. Both methods may be applicable to routine quantitative analyses of promethazine HCl tablet formulations.

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