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Antioxidant assessment on promethazinr HCl decomposition using RP-HPLC assay method

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The objective of this study was to investigate the effect of different sodium metabisulfite (SMBS) concentrations under a variety of ICH recommended test conditions. An attempt was made to test the feasibility of increasing shelf life when stored under different conditions. The promethazine hydrochloride (HCL) sample solutions used according to USP 24 and BP 1999 were prepared using different concentration of sodium metabisulfite as antioxidant. Standard solution was prepared using reference promethazine and analyses were done by employing reversed-phase high-performance liquid chromatography (RP-HPLC). The method used is efficient in acceptable resolution. The effect of different concentrations of SMBS on promethazine was investigated in promethazine HCL degradation. Chemical and physical stability was conducted in different conditions. The result shows that the drug was liable to degradation in basic pH medium condition, though the extent of degradation varied. Separation of the drug and the degradation products under various conditions was successfully achieved. The method was validated and the response was linear (r=0.9998) in the drug concentration range of 5 to 50 µg. The mean values (±RSD) of slope and intercept were 46376 (±0.006975) and 200049 (±0.4009), respectively. The recovery of the drug ranged between 98.3 and 101.16% from the mixture of degradation products. SMBS concentration influences the degradation process. Increase in concentration resulted in decrease of promethazine degradation. The developed method is simple and accurate in use for analysis of the drug and its degradation products. Antioxidant (SMBS) has important role in preventing promethazine degradation beside other factors.

Key words: Degradation, reversed-phase high-performance liquid chromatography (RP-HPLC), promethazine hydrochloride, sodium metabisulfite.

INTRODUCTION

Chemical and physical properties of promethazine HCl

Promethazine hydrochloride (HCl, Figure 1), (2RS)-(10 H-phenothiazine-10-yl) propan-2-amine hydrochloride, is a phenothiazine derivative used as a H-blocker and an antiemetic (Asghar et al., 2011). Promethazine (PROM), like other phenothiazines, is capable of both free-radical and singlet molecular oxygen photosensitization with photoallergic and phototoxic effects. The existence of light-induced free-radicals of some phenothiazines was proven by Forrest (Heyam et al., 2015). Table 1 shows

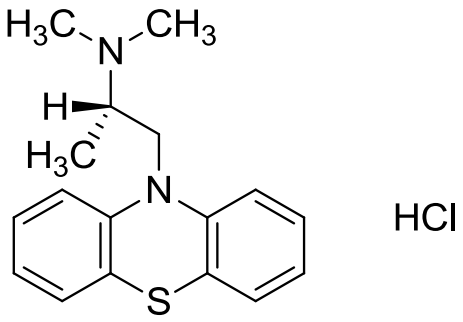

Figure 1. Chemical structures of promethazine HCl.

Table 1. Chemical and Physical properties of Promethazine HCl.

Chemical properties of promethazine hydrochloride substance	
Molecular formula	Promethazine Hydrochloride: $C_{17}H_{20}N_2S.HCl$
Molecular weight	Promethazine Hydrochloride: 320.9
Chemical names	10-[2-(Dimethylamino)propyl]-phenothiazine monohydrochloride
Physical properties of promethazine hydrochloride substance	
Colour	White or faintly yellow. On prolonged exposure to air it is slowly oxidised, becoming blue in color
State/Form	Crystalline powder
Description	-Odourless or almost odourless. -A 10% solution in water has a pH of 4.0 to 5.0 -Solubility 1 in 0.6 of water, 1 in 9 of alcohol, 1 in 2 of chloroform -Practically insoluble in ether and acetone. Melting point is about 222°C. Melting point (promethazine) is about 60°C

the chemical and physical properties of promethazine HCl.

Other characteristics

Shelf-life of the substance, which is the expiry dates of the commercially available parenteral preparation vary between 2 and 5 years. For the storage conditions, all preparations should be protected from light. Oral and parenteral preparations of the drug should be stored at a temperature of 15 to 30°C and freezing of injection should be avoided. The solutions of promethazine should be stored in tightly closed, light resistant containers (Jun et al., 2003).

Antioxidant sodium metabisulfite (SMBS)

Antioxidant plays an important positive role in the stability of promethazine. The concentration of an antioxidant is mandatory in achieving the optimum stability. Therefore, this is investigated in our study. Many antioxidants such as BHA, ascorbic acid and SMBS, and the antioxidant used in this study, all were used in parenteral solutions (Maruchin, 1979). Table 2 shows the chemical and physical properties of SMBS. The synonyms for SMBS are disodium disulfite and disodium metabisulfite (Figure 2).

Effect of antioxidant and degradation

In this case, the presence of promethazine HCl in solution formulations with some additives, like sodium metabisulfite, showed severe degradations if it has been added in insufficient concentration. This will result in insufficient drug's content uniformity, even after short

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Table 2. Chemical and Physical properties of Sodium Metabisulfite (SMBS).

Chemical properties of sodium metabisulfite (SMBS) substance	
Molecular formula	Sodium Metabisulfite $Na_2S_2O_5$
Molecular weight	Sodium Metabisulfite 190.1
Chemical names	Sodium pyrosulfite
Physical properties of sodium metabisulfite substance	
Colour	White to slightly yellow
State/Form	Crystalline power
Description	With an odour of sulfur dioxide. 1 g is soluble in 2 ml of water Decomposes below melting point at 150°C Density: 1.4 g/cm^3 Solubility in water, g/100 ml: 54 (good)
Other characteristics definition	Sodium pyrosulfite contains not less than95.0% of $Na_2S_2O_5$.
Use	As an antimicrobial preservative and as an anti-browning agent

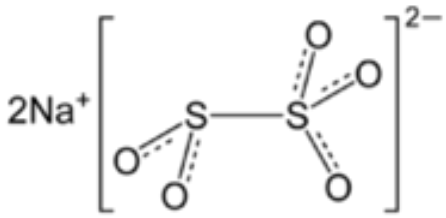

Figure 2. Chemical structures of metabisulfite.

handling periods. The drug degradation is usually accompanied by color changes or precipitation in case the pH has been changed as well, which surely will affect the product's acceptance (Qi et al., 1984).

ICH Stability requirements

Stability requirements for the world wide registrations of pharmaceutical products have undergone a dramatic change in the past few years with the advent (introduction) of the guidelines of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). ICH has introduced a standardized approach for the development of stability data for registration through various guidelines (Heyam et al., 2015; Sane et al., 2008). From these requirements, it is necessary to develop a stability indicating assay method for these drugs to ensure the safety, quality and efficacy of these drugs. “Stable”, as used in the context of this application, means remaining in a state or condition that is suitable for administration to a patient. Formulations according to the present formulations are found to be stable when maintained at room temperature for at least 12 months and are generally stable at room temperature for 12 to 24 months. In laboratory studies, it has been found that precipitates are formed when promethazine hydrochloride solution is exposed to light and air; therefore, to be kept away from light under storage condition is mandatory. Also, it is believed that this precipitate formation could be related to change of pH and trace metals found in water. In this study, the stability of a standard promethazine HCl solution after storage at room temperature and under refrigerated conditions was monitored. In an effort to extend the shelf life of this formulation, an antioxidant, a buffering agent, or both were added to the solution; the drug stability in the modified formulations was examined (Sane et al., 2008; Stavchansky et al., 2011). Therefore, the objective of this study was to investigate the stability of promethazine HCl injectable solution using different concentrations of SMBS as antioxidant. To investigate the effect of different conditions on promethazine degradation under a variety of ICH recommended test conditions. And an attempt to test the feasibility of increasing its shelf life when stored at different conditions (Miller et al., 2010).

EXPERIMENTALS

Materials and reagents

All chemicals used were of analytical reagent grade. Promethazine reference and SMBS were provided from Julphar Company. Promethazine HCl, sodium meta-bi-sulphite, sodium EDTA (disodium edentate), glacial acetic acid, sodium acetate tri-hydrate, and water were preparation and used for injection. Promethazine

Table 3. Promethazine HCl sample formulations containing different SMBS concentrations.

Formulation composition (mg/ml)	F0	F1	F2	F3	F4
Promethazine HCl	25.4	24.8	25.06	24.9	25.5
SMBS	0.250	0.292	0.340	0.382	0.300
Disodium Edetate	0.1	0.1	0.1	0.1	0.1
Sodium acetate trihydrate	2.25	2.25	2.25	2.25	2.25
Glacial acetic acid	0.6294	0.6294	0.6294	0.6294	0.6294
Water for injection	Q.S to 1000	Q.S to 1000	Q.S to 1000	Q.S to 1000	Q.S to 1000

HCL standard, pentansulfonic acid sodium salt, glacial acetic acid, acetonitrile reversed-phase high-performance liquid chromatography (RP-HPLC) grade, and ultra-pure water were used for the assay of promethazine.

Methods

In this work, the proposed method was applied using an RP-HPLC method for the determination of PROM HCl in its injection drug formulation and is based on its oxidation by coupling with sulfanilic acid sodium (Arnao et al., 2012). Chromatographic conditions: Column, L11- (µ bondapak phenyl 125 A 10 µm 300 X 3.9 mm) (waters); Mobile phase: mixture of ion pair solution and acetonitrile in ratio 50:50 (v/v); Flow rate: 1.0 ml/min; Detection: UV 254 nm; Injection volume: 20 µl; Temperature: ambient; Diluent: 0.1% glacial acetic acid in water. Preparation of ion pair solution: Wight and transfer of 2 g of 1-Pentansulfonic acid sodium salt in 1000 ml volumetric flask, dissolve with 900 ml water, and addition of 10 ml glacial acetic acid and complete to the volume with water filtered through 0.45 µm filter paper (Jinjiang and Yongmei, 2014).

Identification of promethazine HCl

For the preparation of promethazine standard solutions, about 50 mg of promethazine HCl standard were weight and transfer accurately into a 100-ml volumetric flask, dissolve and make up to volume with diluent. 5.0 ml of the resulting solution pipetted and transferred accurately into a 100.0 ml volumetric flask, diluted to volume with diluent (this is a standard solution containing 25.0 µg/ml of promethazine HCl). For the preparation of test solution, 2.0 ml of test injection were pipetted and transferred accurately into a 100 ml volumetric flask, dilute to volume with diluent and well shake (solution A). 5.0 ml of solution A was pipetted and transferred accurately into a 100 ml volumetric flask, diluted to a volume with diluent, and well shake (this is test solution for assay containing claimed concentration about 25 µg/ml promethazine HCl) (Cano and Hernandez-Ruiz, 1998). Table 3 shows promethazine HCl sample formulations containing different SMBS concentrations.

System suitability

Make 6 replicate injection of standard solution and measure the peak area. The relative standard deviation of the replicate injection is not more than 2.0%.

RP-HPLC identification

The chromatogram of the test preparation exhibits a major peak, the retention time of which corresponds to that of the promethazine HCl peak in the chromatogram of the standard preparation (Alcolea et al., 2002; Cano et al., 2000). 20 µl of standard solution were injected separately and the solution was tested for the assay into the chromatograph. The chromatograms were recorded and the peak areas were measured. For the assay of promethazine HCl, the quantity was calculated, in mg, of promethazine HCl in each ml of injection by the formulas (Pulido et al., 2003):

$$PAt \times C \times P \times DF / PAs \times 1000$$

where Pat is the peak area of promethazine HCl in the chromatogram of test solution, Pas is the peak area of promethazine HCl in the chromatograph of the standard solution, C is the concentration of promethazine HCl (µg/ml) in the standard solution, P is the potency of promethazine HCl standard, and DF is the dilution factor (1000).

RESULTS

Promethazine HCl standard solution formulation specifications were determined initially as summarized in the Table 4. Promethazine HCl sample solution was formulated in different conditions (dark and light) at room temperature in amber glass bottles.

RP-HPLC analysis was conducted in different intervals according to ICH. The obtained chromatograms are represented in Figures 3 to 7. Figure 4 shows a typical chromatogram of promethazine HCl in standard solution. Figure 5 shows a typical chromatogram of promethazine HCl in the test solution. Figure 6 shows a typical chromatogram of promethazine HCl and its related substance, and finally, Figure 7 shows a typical chromatogram of the reference solution. The calibration curve of the chromatograms values of promethazine concentration vs. area under the curve in mAU were plotted (Figure 3).

The recovery of promethazine formulations were calculated (Table 5). The recovery calculated by dividing the added value divided by the found value in percentage.

The results of the short term physical and chemical stability studies conducted on promethazine HCl injectable solutions are shown in Tables 6 and 7, respectively. It can be observed from the results that formulations protected from light were more stable than the ones exposed to light. Formulations containing high amount of SMBS concentrations showed more stability. The samples of promethazine HCl showed an acceptable

Table 4. Specifications of reference promethazine HCl 25 mg/ml injection.

Test parameter	Specifications
Physical	
I-Description	Clear, color less solution
II-PH	Between 4.0 and 5.5
Chemical	
I-Identification	The retention time of the major peak in the chromatogram of the assay preparation corresponds to that in the chromatogram of the standard preparations, as obtained in the assay
II-Assay	25 mg/ml
Promethazine hydrochloride	Limit: Not less than 95% and not more than 110% of the labeled amount of $C_{17}H_{20}N_2S.HCl$
Sodium metabisulphite	Limit: 0.10 - 0.30 mg/ml

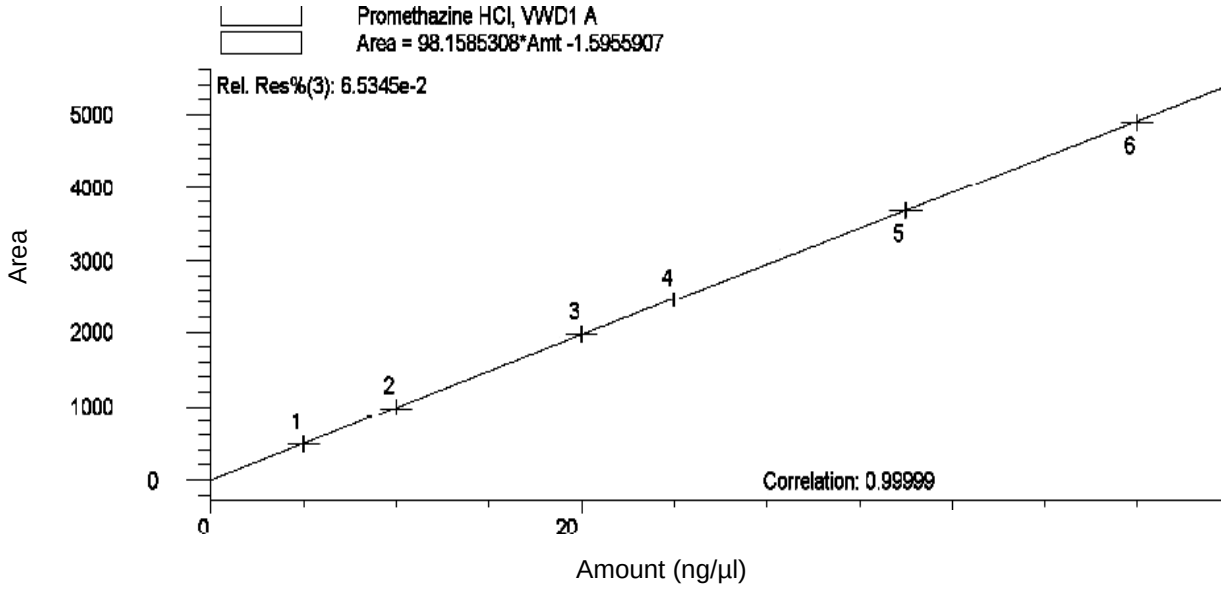

Figure 3. Calibration curve of promethazine.

physical and chemical stability of up to 3 months when stored at room temperature protected from light using all the concentrations (Nielsen et al., 2003). The addition of SMBS gave a concentration <300 mg/ml when stored and protected from light. The results showed a higher stability for all formulations after storage at room temperature protected from light compared with those stored in exposure to light.

DISCUSSION

Promethazine decomposition mechanism, rapid decomposition of promethazine HCl solution under the influence of day-light, air and trace elements in water led to the loss of an electron to yield the semi-quinone free radical (PROM●). Promethazine light + air in H_2O (PROM●) semiquinone free radical. Further degradation produces phenazathionium ion (PROM●+), (PROM●) (PROM●+), and phenazathionium ion will react with water to give the corresponding sulfoxide [PROM (S-O)], phenazathionium ion H_2O [PROM (S-O)] sulfoxide (Pietta et al., 2000).

Dimerization may occur as a result of terminating the monomers free radicals, as follows:

2 (PROM●) DimerizationPROM - PROM (2 PROM), (semiquinone free radical) (terminated dimer).

There was no color change, precipitation, or other visible sign of incompatibility at any time during the study (Borkar et al., 2009). Promethazine hydrochloride and

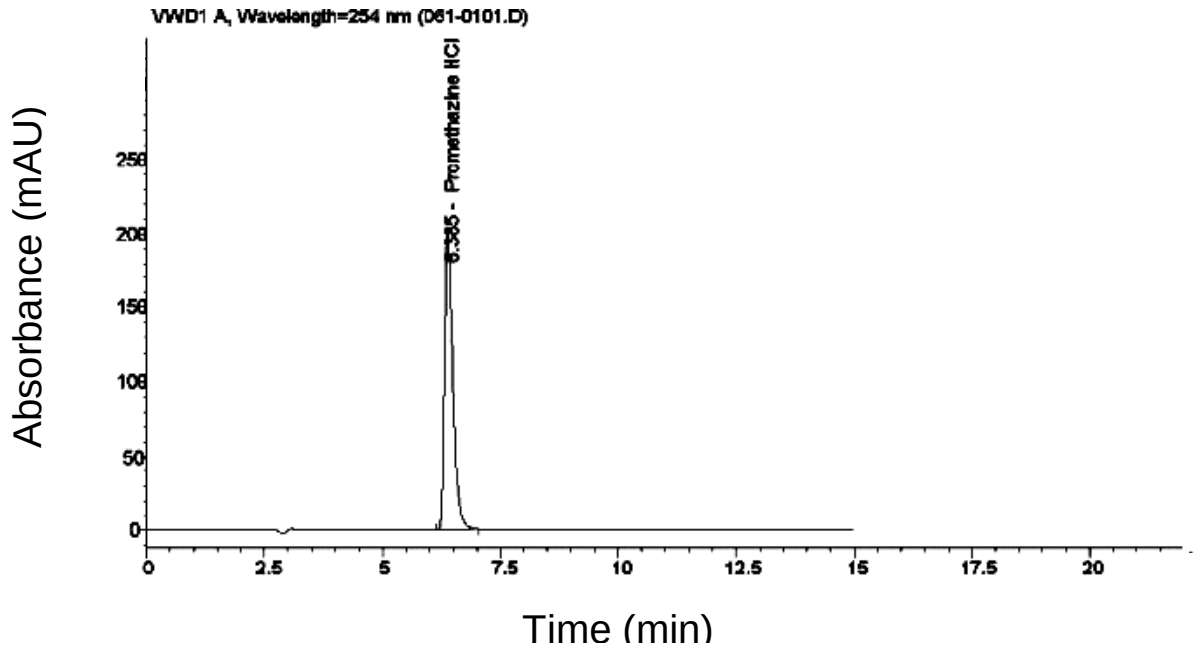

Figure 4. Typical chromatogram of promethazine HCl in standard solution.

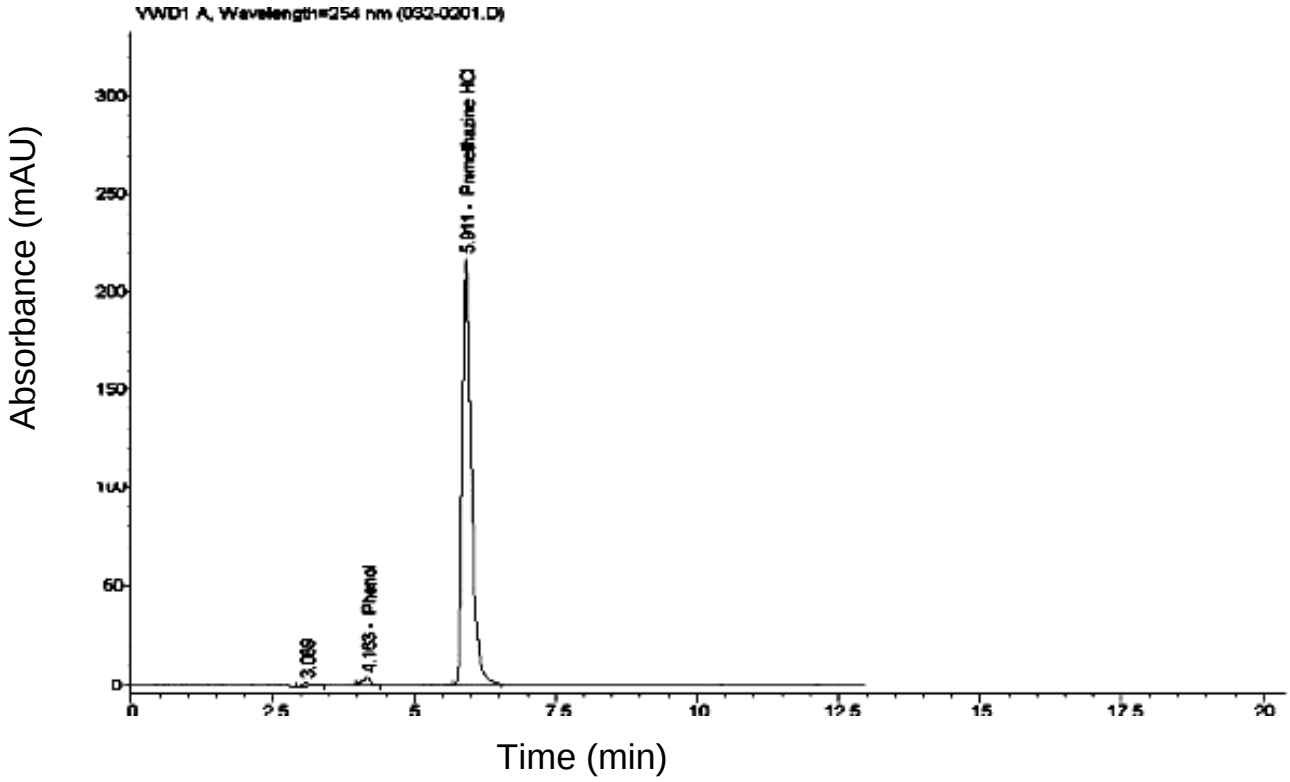

Figure 5. Typical chromatogram of promethazine HCl in test solution.

SMBS remained stable under all study conditions (Table 4). The stability of PROM in water is mediated by the rate of acid/base hydrolysis (Cao et al., 1998). The rate of degradation of PROM hydrochloride has been reduced

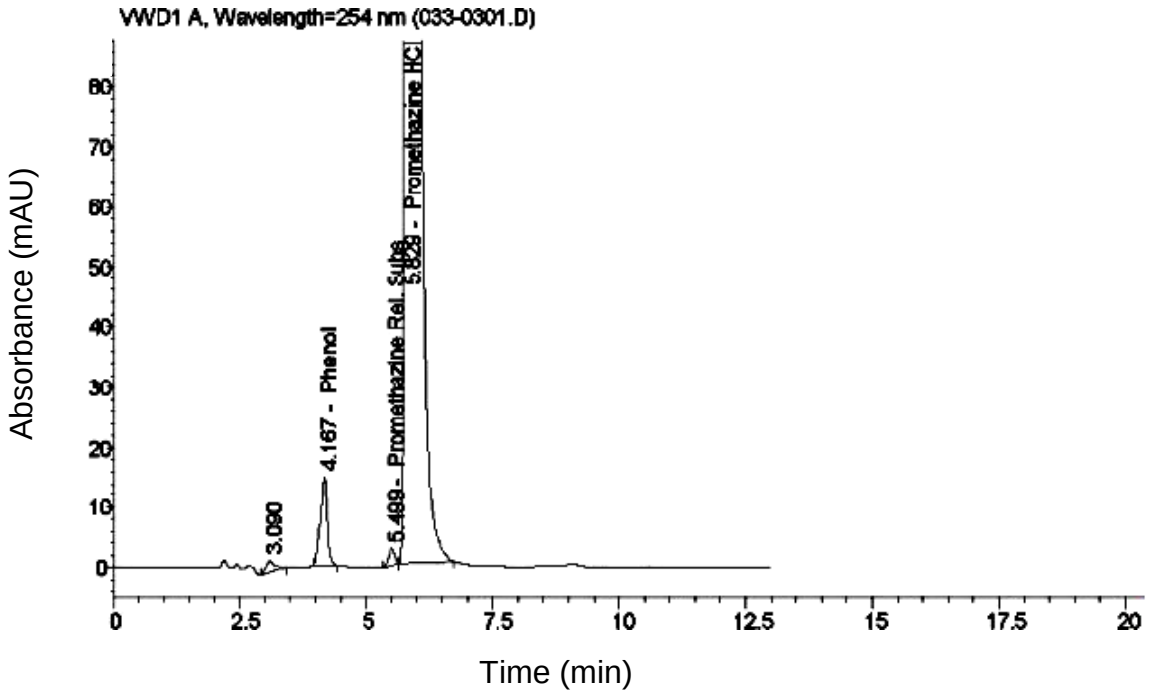

Figure 6. Typical chromatogram of promethazine HCl and its related substance.

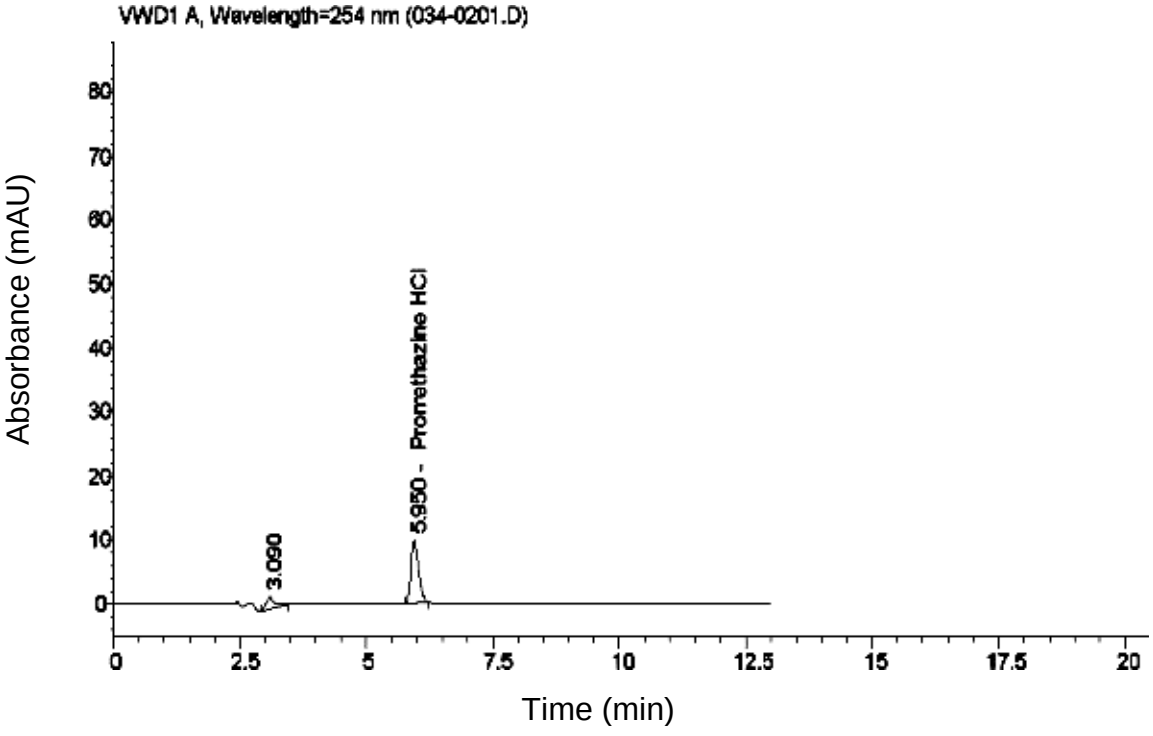

Figure 7. Typical chromatogram of the reference solution.

by the use of acetate as a buffer, maintaining the pH as close to 5.0 as possible, minimizing the hydrolysis reaction if the buffer used. The result of this study showed that the drug was liable to degradation in all

Table 5. % Recovery of promethazine formulations.

Parameter	Added* (µg)	Found (µg)	Recovery (%)
F0	25.4	24.9	98.03
F1	24.8	24.7	99.55
F2	25.06	24.8	98.96
F3	24.9	24.8	99.59
F4	25.5	25.4	99.60

Masses of standard promethazine hydrochloride added in formulations (F0 to F4).

Table 6. Physical stability of promethazine HCl solutions for three month in dark and light exposure.

Parameter	pH			Appearance		
	1st Month	2nd Month	3rd Month	1st Month	2nd Month	3rd Month
F0						
Light	4.6	4.8	.4.8	NC	NC	NC
Dark	4.5	4.5	4.6	NC	NC	NC
F1						
Light	4.6	4.8	4,9	NC	NC	NC
Dark	4.8	4.8	4.9	NC	NC	NC
F2						
Light	5.1	5.1	4.9	NC	NC	NC
Dark	4.8	4.7	4.6	NC	NC	NC
F3						
Light	4.6	4.8	4,9	NC	NC	NC
Dark	4.8	4.8	4.9	NC	NC	NC
F4						
Light	4.8	4.8	4.9	NC	Precipitate	Precipitate
Dark	5.1	5.1	4.9	NC	NC	NC

NC: No color change; D: darkening (solution); DS: slight darkening in color.

Table 7. Chemical stability of promethazine HCl 25 mg/ml formulations[a] stored in light and dark **c**onditions.

Storage condition at room temperature	Actual initial promethazine concentration (mg/ml)	SD % Initial concentration remaining		
		Mean ± 1st Month	Mean ± 2nd Month	Mean ± 3rd Month
F0				
Light	24.7 ± 0.003	99.5 ± 0.4	97.5 ± 0.2	94.5 ± 0.2
Dark	24.7 ± 0.004	101.0 ± 1.1	100.6 ± 1.1	99.8 ± 1.2
F1				
Light	24.8 ± 0.005	95.6 ± 0.3	94.5 ± 0.2	93.3 ± 0.5
Dark	24.7 ± 0.003	99.5 ± 0.4	98.6 ± 0.3	98.6 ± 0.2
F2				
Light	24.9± 0.004	98.9 ± 8.9	97.9 ± 7.8	96.9 ± 5.8
Dark	24.7 ± 0.005	99.9 ± 0.8	99.5 ± 0.6	98.5 ± 0.6

Table 7. Contd.

F3				
Light	24.9 ± 0.003	99.9 ± 0.8	98.5 ± 0.6	97.5 ± 0.5
Dark	24.8± 0.004	100.6 ± 0.3	100.5 ± 1.2	99.8 ± 1.2
F4				
Light	25.4± 0.004	96.9 ± 8.9	93.9 ± 7.8	90.9 ± 5.8
Dark	24.7 ± 0.005	99.9 ± 0.8	98.5 ± 0.6	975 ± 0.6

[a]Mean ± SD calculated from triplicate assays on single samples from each container. Samples were either protected from light by using aluminum foil or exposed to fluorescent light.

condition, though the extent of degradation varied (Awika et al., 2003). The promethazine HCl was found to be more liable to decompositions in alkaline solution than in acidic solution and neutral condition. Degradation of promethazine HCl in neutral solution was observed relatively after long hour of refluxing indicating that the drug is relatively stable in neutral condition (Rasha et al., 2014).

Conclusion

This method was specific to the drug and also selective to degradation products. The developed method is simple accurate, precise, specific and selective and rugged and thus it can be used for analysis of the drug and its degradation products. Antioxidant (SMBS) has important role in preventing promethazine degradation. Samples of promethazine HCl contained an acceptable drug concentration for up to three months when stored at room temperature protected from light (Leclercq et al., 2000). The addition of SMBS at a concentration of 0.29 to 0.38% w/v to the promethazine formulations increased the suspension's shelf life at room temperature when stored protected from light (Bonilla et al., 1999).

Conflict of Interests

The authors have not declared any conflict of interests.

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