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Development and validation of a HPLC method for the analysis of promethazine hydrochloride in hot-melt extruded dosage forms

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A simple and rapid stability-indicating HPLC method was developed for determination of promethazine hydrochloride (PMZ) in hot-melt extruded (HME) films and sustained release tablets. Chromatographic separation was achieved on a 150 mm × 4.6 mm i.d., 3 μm particle size, C8 (2) column with acetonitrile-25mM phosphate buffer (pH 7.0), 50 : 50 (v/v) as mobile phase at a flow rate of 1 mL · min^{-1}. Quantitation was achieved with UV detection at 249 nm based on peak area. The method was validated in terms of linearity, precision, accuracy, robustness specificity, limits of detection and quantitation according to ICH guidelines. Specificity was validated by subjecting the drug to acid, base, oxidative, reductive and dry heat degradations. None of the degradation products obtained by forced degradation interfered with the PMZ peak. The method was successfully applied for assessing the stability of the drug in the HME films and sustained release tablet formulations. In addition, uniformity of PMZ content in HME films was also determined using the method developed. Excipients present in either of the dosage forms analyzed did not interfere with the analysis indicating the specificity of the method. Due to its simplicity and accuracy, the method is suitable for application to various dosage forms.

1. Introduction

Promethazine hydrochloride (PMZ) is a potent histamine H1-receptor antagonist used in a variety of clinical situations: for the prevention and treatment of nausea and vomiting caused by narcotic therapy, migraine and cancer chemotherapy; as a sedative, particularly as a part of a preoperative drug regimen and during labor; and for motion sickness (United States Pharmacopeial Convention 1996). Commercially, PMZ is available in the tablet, syrup, injection and suppository dosage forms. All of the commercially available dosage forms are official in the United States Pharmacopeia (United States Pharmacopeia 2003).

For prophylaxis of nausea and vomiting, as during surgery and the postoperative period, and for treating motion sickness, the drug has to be administered frequently (3–4 h interval as needed). Hence, a controlled delivery system would be preferred to reduce the frequency of dosing and the side effects associated with it. Also, when PMZ cannot be tolerated orally, injections and suppositories are the only options. However, each of these routes has their own disadvantages. An attempt has therefore been made to deliver the drug through an alternate route i.e. oral transmucosal route via incorporation into polymeric films produced by the hot-melt extrusion (HME) technology. The oral mucosal route offers some unique advantages including avoiding the first pass effect, easy accessibility and enhanced patient compliance.

The wavelengths of maximum absorption for promethazine are 249 and 298 nm. Most of the analytical methods using HPLC to determine PMZ were aimed at quantifying the drug in plasma (Jang et al. 2005; Strenkoski Nix et al. 2000; Vanapalli et al. 2001). Only two stability-indicating HPLC methods using UV detection are available in the literature for determination of PMZ in various dosage forms such as tablets, syrups and suppositories (Mathew et al. 1994; Yang et al. 1985). However, neither of these methods was found to be reproducible for the determination of the active drug remaining during the stability studies in the HME films and sustained release buccal tablets. Moreover, a number of excipients present in these dosage forms such as polymers, bioadhesives, plasticizers and other excipients produced interference with the active peak at the wavelengths used.

The purpose of this investigation was to develop a stability-indicating HPLC method that could be routinely employed to assess the stability and content uniformity of the

drug in HME films and also for quantifying the compound in various other pharmaceutical dosage forms. The developed method was validated according to ICH guidelines. Forced degradation studies performed on the drug indicated the specificity and stability-indicating nature of the method. None of the excipients utilized produced interference with the drug peak indicating the suitability of this method for routine determination of the drug in various pharmaceutical dosage forms.

2. Investigations, results and discussion

2.1. HPLC method development

An attempt was made to develop a method that can be used for determination of PMZ in hot-melt extruded polymeric films and sustained release tablets. An aqueous solution of pure drug ($40\ \mu g \cdot mL^{-1}$) and a degraded sample of the drug were used as samples for method development. A mobile phase consisting of water: methanol

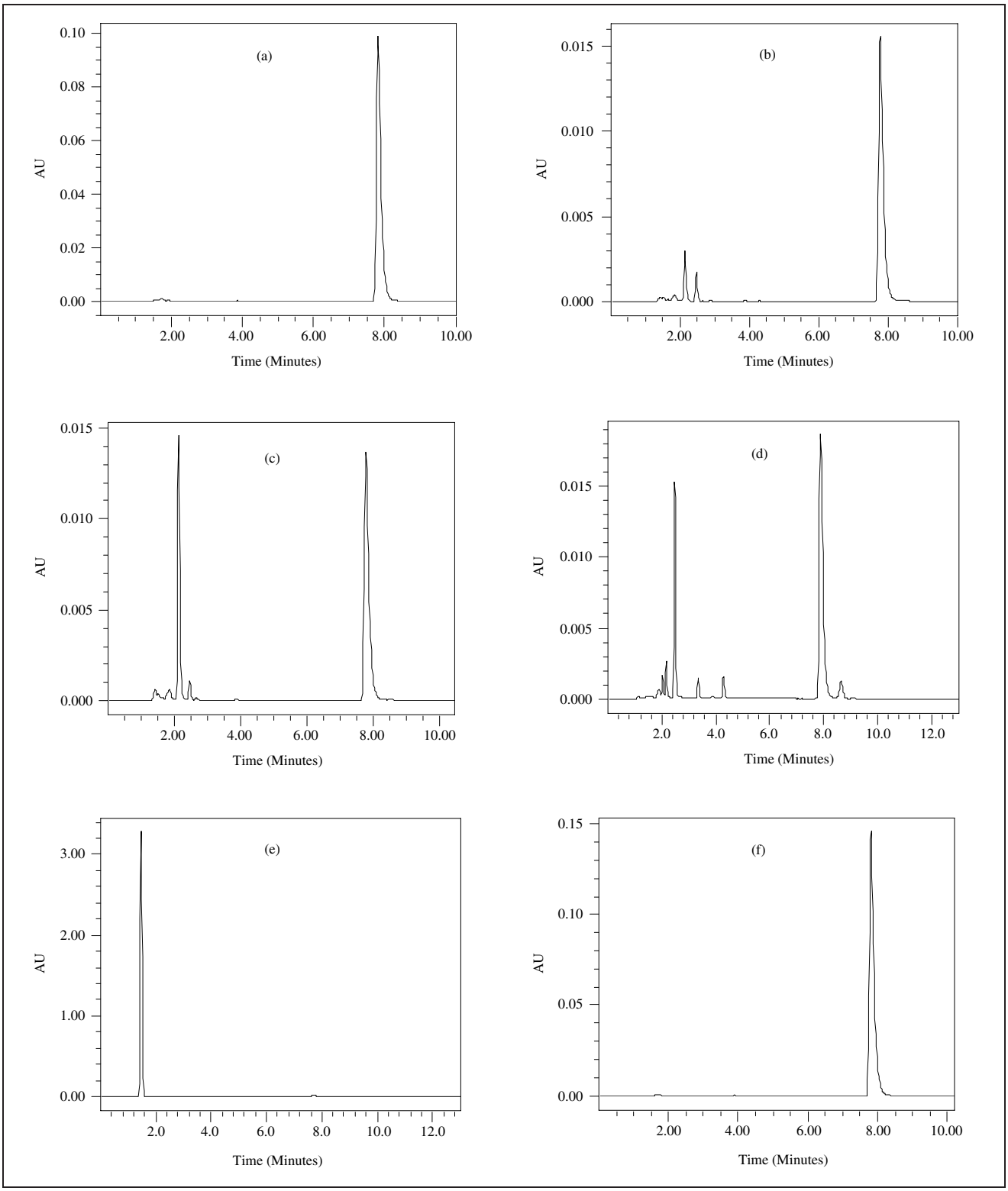

Fig. 1: Chromatograms of (a) pure PMZ, PMZ solution subjected to (b) acid hydrolysis, (c) alkaline hydrolysis, (d) neutral hydrolysis, (e) reduction and (f) thermal stress

(30 : 70, 50 : 50 and 70 : 30 v/v) did not result in effective mobility for acceptable analysis of PMZ, which could not be detected. A broad drug peak at an R_t of 12 min was observed with a mobile phase composition consisting of 70 : 30 (v/v) ACN: water at a flow rate of 1 mL · min^{-1}. Increasing the flow rate to 1.5 mL · min^{-1} at this composition although, decreased the retention time of the drug to 9 min, did not improve the peak shape. Decreasing the ACN phase to 40% v/v increased the retention time of the drug to 15 min. The peak obtained in this case was broad and showed tailing. A mobile phase consisting of 50 : 50 v/v ACN: 25 mM dibasic potassium phosphate buffer (adjusted to pH 7.0 using o-phosphoric acid), at a flow rate of 1 mL · min^{-1} resulted in a well defined peak with an acceptable retention time of 7.78 min. A typical chromatogram of the pure PMZ sample obtained using the optimized method is shown in Fig. 1a. Tailing factor for PMZ was less than 2 and the resolution of the analyte in presence of degradation products was satisfactory. The method thus developed was further validated.

2.2. Method validation

The *LOD* of the analyte was found to be 0.1 μg · mL^{-1} at three times the baseline noise. *LOQ* was determined to be 0.5 μg · mL^{-1} at 10 times the peak-to-peak noise with a % RSD of 0.21% for three successive injections of the sample. The *range* of detection of the analyte for the HPLC method developed was found to be from 0.1 to 250 μg · mL^{-1}.

A calibration curve constructed by plotting the mean PMZ peak area against concentration was *linear* in the range of 25–250 μg · mL^{-1}. Peak area and concentration were subjected to linear least-squares regression analysis to obtain the calibration equation and coefficient of determination. The regression equation was found to be

$$Y = 8418\ X + 7894\ (r^2 = 0.999,\ n = 6;\ Y = mX + c)$$

where Y is the area of the PMZ peak, m is the slope, c is the intercept and X is the concentration (μg · mL^{-1}) of the solution injected. The statistical parameters including correlation coefficient, slope and y-intercept are presented in Table 1. There was little variation in the slope and coefficient of determination values for all of the calibration samples injected on different days. Also, the coefficient of determination was found to be greater than 0.999 in all of the cases indicating that the method is very linear over the range tested.

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the true value. The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (ICH 2005). The results of *intra-day accuracy* and precision are presented in Table 2. Intra-day precision was calculated from the percent variation within the replicate injections of the controls. The intra-day accuracy varied from −2.26 to 1.05%, indicating that the samples were stable for at least 12 h in a single day. The intra-day precision varied from 0.14 to 0.97%. *Inter-day accuracy* and *precision* values of the controls analyzed varied from −1.01 to 0.29% and 0.38 to 0.40%, respectively (Table 3). Thus the method developed proved to be accurate and precise.

The *robustness* of the method was evaluated by changing some of the key system parameters, such as mobile phase composition, pH and flow rate (Table 4). The selected factors remained unaffected by small variations of the parameters studied. The values obtained for Rs, T and N were well within the acceptable limits of system suitability testing (Carstensen and Rhodes 2000). Also, the variation in peak areas was insignificant. None of the variables had a significant effect on % RSD of the replicates. The resolution was found to be > 2 with all of the variables studied,

Table 1: Linearity data of promethazine hydrochloride determination

Day	Slope	Y-intercept	Coefficient of determination (r^2)
1	8507	12699	0.9997
2	8491	13422	0.9999
3	8511	12643	0.9995
4	8259	15235	0.9990
5	8404	11233	0.9991
6	8497	14342	0.9993
Mean	8445	13262	0.9994
SD	99	1407	0.0003

Table 2: Intra-day accuracy and precision of the method (n = 3)

Time (h)	Concentration known (μg · mL^{-1})	Concentration found (μg · mL^{-1})	% Deviation (Accuracy)	% RSD (Precision)
0	40	39.11	−2.22	0.49
	75	75.59	0.79	0.77
	125	123.92	−0.87	0.62
6	40	40.42	1.05	0.14
	75	74.63	−0.49	0.18
	125	124.33	−0.54	0.53
12	40	39.09	−2.26	0.21
	75	74.38	−0.82	0.97
	125	123.61	−1.11	0.56

Table 3: Inter-day accuracy and precision of the method (n = 3)

Concentration known (μg · mL^{-1})	Concentration found (μg · mL^{-1})	% Deviation (Accuracy)	% RSD (Precision)
40	39.6	−1.01	0.38
75	75.2	0.29	0.44
125	124.2	−0.53	0.40

Table 4: Robustness with respect to three critical method parameters

Method parameter	Rs[a]	T[b]	N[c]
Mobile phase pH			
6.8	5.59	1.1	2762
7.0	3.11	1.0	2715
7.2	3.07	1.32	3309
Percent acetonitrile			
48	4.37	1.5	3650
50	3.41	1.0	3215
52	3.06	1.25	2334
Flow rate (mL · min^{-1})			
0.8	2.56	1.26	4082
1.0	3.21	1.0	2785
1.2	4.60	1.48	4328

[a] Resolution
[b] Tailing factor
[c] Number of theoretical plates

suggesting the stability-indicating nature of the developed method. The analyte peak was found to be very symmetric indicated by its low tailing factor value ($T < 2$). Thus the developed method was found to be robust.

During the development of stability-indicating methods, when little or no information is available regarding the potential degradation products, forced degradation is performed to demonstrate the *specificity.* These studies also provide information about degradation pathways and degradation products that could form during storage so that measures can be taken during formulation to avoid potential instabilities (Önal et al. 2007). An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products.

Degradation by acid. The chromatogram of the pure drug is depicted in Fig. 1a. PMZ eluted at a retention time of 7.78 min. The peak obtained was very symmetric and showed no interfering peaks. On heating the drug solution in 1 N HCl at 80 °C for 2 h, partial degradation of PMZ was observed. Three additional peaks were observed well separated from PMZ peak at retention times of 1.84, 2.13 and 2.47 min (Fig. 1b).

Degradation by base. On subjecting the drug to basic hydrolysis at 80 °C for 2 h, the drug showed degradation in the form of three additional peaks in the chromatogram, all eluting in less than 4 min (Fig. 1c). The degradation peaks were well resolved from the analyte peak and had retention times of 1.85, 2.13 and 2.47 min.

Neutral hydrolysis. Significant degradation was observed on heating an aqueous solution of drug. The peak area of PMZ was reduced considerably and seven additional peaks were observed in the chromatogram (Fig. 1d). None of the degradation peaks showed any interference with the drug peak, which was found to be symmetric ($T < 2$).

Degradation by reduction. When the PMZ solution was exposed to 10% $NaHSO_3$ for 10 min, the chromatographic peak corresponding to the drug ($Rt = 7.7$ min) disappeared completely and a new peak at a Rt of 1.45 min (attributed to $NaHSO_3$) was observed (Fig. 1e). No other degradation products were observed in the chromatogram of the degraded sample.

Degradation by dry heat. When the solid drug was subjected to dry heat at 80 °C for 3 days, no decomposition of the drug was observed (Fig. 1f). This indicates that the drug is very stable in solid-state and shows significant degradation in solution state.

Degradation by oxidation. Oxidative degradation of the drug sample was studied by refluxing the sample with 3% (v/v) H_2O_2 solution for predetermined time intervals of 0, 5 and 15 min. The drug was found to degrade very rapidly in presence of H_2O_2 (Fig. 2). At time zero, PMZ showed immediate degradation with two additional peaks at retention times (R_t) of 1.72 and 2.15 min. The peak at 1.72 min may be attributed to H_2O_2. Upon increasing the heating exposure, the peak height of the drug decreased and that of the degradant peak at 2.15 min increased continuously. The drug degraded completely after 15 min. The results of this study clearly indicated that the proposed method is specific and has a stability-indicating nature.

The purity of the drug peak in all of the stressed sample solutions (except the sample degraded by reduction) was determined utilizing a photodiode array detector (PDA) (Fig. 3). Peak purity measurements confirmed that the

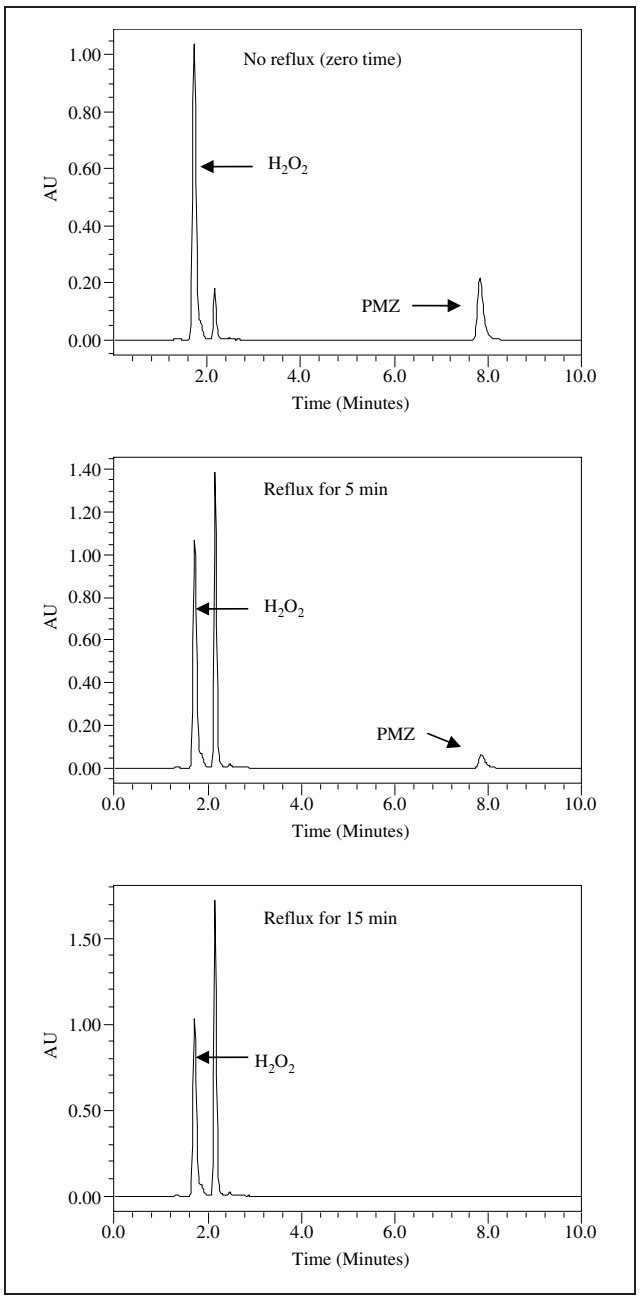

Fig. 2: HPLC chromatograms of PMZ solution subjected to oxidative degradation with 3% (v/v) H_2O_2, showing decreasing peak height of the drug with increasing heating duration

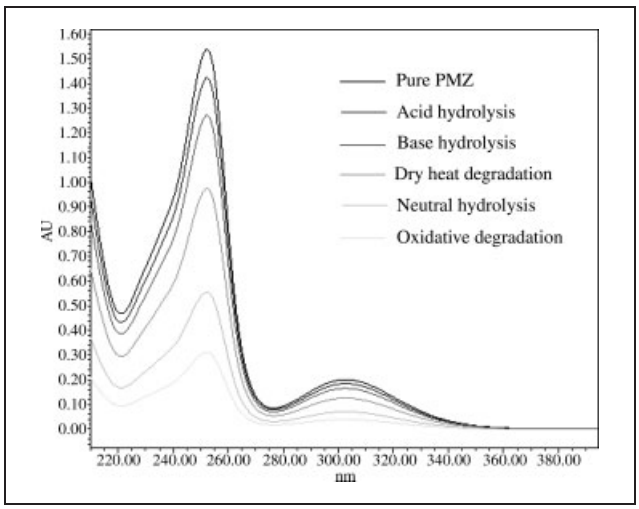

Fig. 3: Photo-diode array spectra of the chromatographic peak corresponding to promethazine hydrochloride after subjecting the samples to forced degradation

peak is homogeneous and pure in all of the stressed samples analyzed. No coelution of any of the degradant peaks was observed in the extracted UV spectra of PMZ peak in all of the stressed samples.
Solutions injected after 10 days of storage at room temperature failed to show any appreciable change in drug amount. No additional peak was observed in the chromatograms of the analyzed samples indicating the stability of PMZ in sample solutions.

2.3. Analysis of promethazine hydrochloride in tablets and HME films

Typical chromatograms obtained for the tablet formulation and the HME film at 6 month stability time points are presented in Fig. 4. These chromatograms show that the excipients used in either of the dosage forms did not interfere with the active drug. Also, the degradation products were well resolved from the active compound indicating that the method is specific. The percent PMZ remaining at the end of the study was found to be 80.9 ± 1.56 and 94.8 ± 2.12 from tablet formulation and HME films, respectively via HPLC method (Fig. 5). The HME films analyzed exhibited a post-processing drug content of 99.5 ± 1.23. The low standard deviation value indicated that the distribution of drug is uniform through out the HME film. Thus the developed method was successfully applied for assessing the stability of PMZ in various pharmaceutical dosage forms and also for content uniformity measurements.

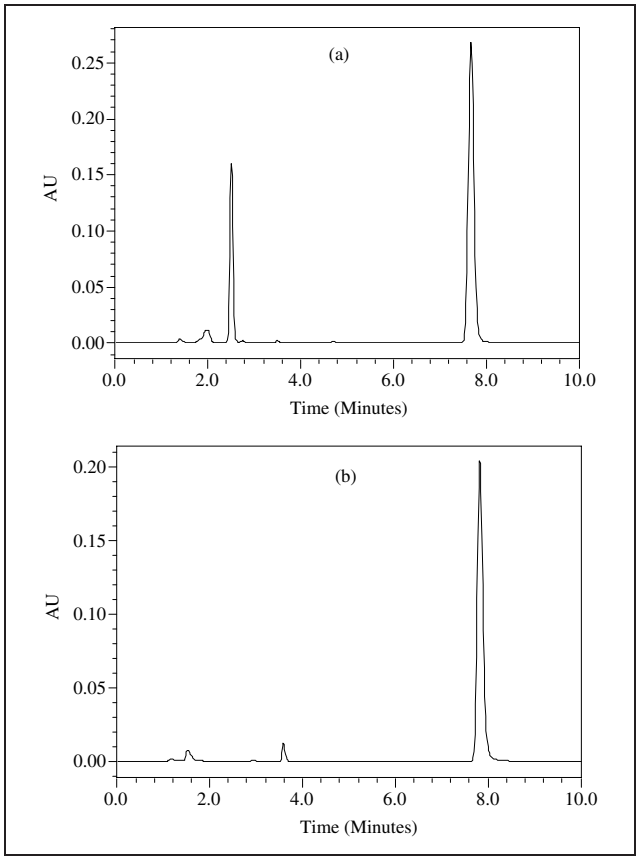

Fig. 4: Representative chromatograms of PMZ from (a) the tablet formulation and (b) the hot-melt extruded films at the 6 month stability time point

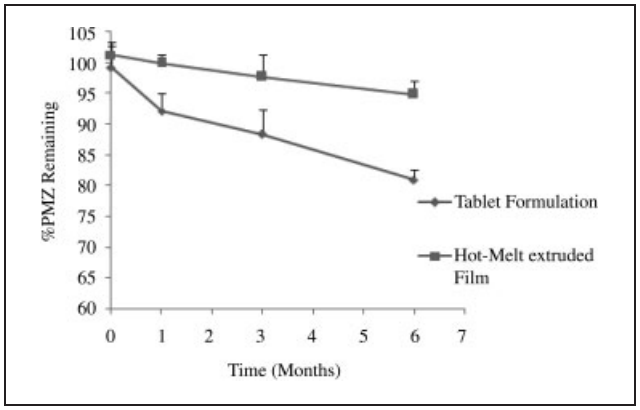

Fig. 5: Stability of promethazine hydrochloride in the sustained release tablets and the hot-melt extruded films determined via the HPLC method developed. The samples (n = 3) were stored at 40 °C/75% RH

3. Experimental

3.1. Materials

Promethazine hydrochloride was obtained from Hawkins, Inc., (Minneapolis, MN). Methanol (HPLC grade), acetonitrile (HPLC grade), *o*-phosphoric acid, potassium phosphate dibasic, hydrochloric acid (37.3%), sodium hydroxide (98.4%) and hydrogen peroxide (3% w/w) of reagent grades were obtained from Fischer Scientific (Fair Lawn, NJ). Sodium bisulfite (purity $\geq$ 99%, reagent grade) was purchased from Sigma-Aldrich (St. Louis, MO). Double distilled nanopure water was obtained in-house from a Millipore water purification unit.

3.2. Chromatographic conditions

A Waters HPLC-UV system (Waters Corp, Milford, MA) and a Luna 3μ C-8 (2), 150×4.60 mm reversed-phase column (Phenomenex, Torrance, CA), were used at a detection wavelength of 249 nm. The mobile phase consisted of acetonitrile (ACN)-25mM dibasic potassium phosphate (50 : 50, vol./vol., adjusted to pH 7.0 with orthophosphoric acid), which was degassed prior to operating under isocratic conditions. The analyte was detected using a Waters 2917 detector. The flow was maintained at 1.0 mL · min^{-1}, with PMZ and degradants eluting within 10 min. The temperature of the column was maintained at 25 °C. Sample injection volume was 20 μL. A calibration curve was constructed for the drug using a series of standard solutions of known concentrations, and the area under the peak was employed to determine the concentration of PMZ in the sample solutions.

3.3. Method validation

3.3.1. Preparation of standards and controls for method validation

Five calibration standards and three controls were used for method validation. A stock solution of 250 μg · mL^{-1} was prepared by dissolving an appropriate amount of the drug in methanol. Other calibration standards (150, 100, 50 and 25 μg · mL^{-1}) were prepared by serially diluting the stock solution. Three controls (125, 75 and 40 μg · mL^{-1}) were prepared so as to lie within the range of the standard calibration curve. The calibration standards and control samples were prepared from separate stock solutions.

3.3.2. Limit of detection (LOD), limit of quantitation (LOQ) and range

The concentrations of the analyte with 3 and 10 times the base line noise were taken to be the LOD and LOQ, respectively, of the drug (Foppa et al. 2007). Methanol (blank) with out any analyte was injected to determine the peak-to-peak noise. LOD and LOQ were experimentally determined by serially diluting a 250 μg · mL^{-1} concentration of the analyte until the average absorbances of three replicates were 3 and 10 times, respectively of the base line noise. The range of detection of PMZ was determined from the lowest detectable concentration to the highest concentration tested.

3.3.3. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample (ICH 2005). Linearity of the method was determined by injecting the five calibration standards (250, 150, 100, 50 and 25 μg · mL^{-1}) in triplicate for six consecutive days. A calibration curve was constructed by plotting the mean peak areas of the analyte against the concentration. Linear regression analysis was performed and the calibration equation of the trendline is used to obtain the coefficient of determination, slope and y-intercept.

3.3.4. Intra-day and inter-day accuracy and precision

To evaluate the intra-day and inter-day accuracy and precision, three replicate injections of five calibration standards and three controls were made

at an interval of 6 h on the same day and for 7 consecutive days, respectively. The concentration of these controls was estimated from the linear regression line of the calibration standards. The percent deviation of the calculated concentrations of the controls from the actual concentrations was used to calculate the intra-day and inter-day accuracy. The variation within replicates (expressed as % RSD) was used to calculate intra and inter-day precision (Avery et al. 1999).

3.3.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in some parameters and provides an assurance of its reliability during normal usage (ICH 2005). To evaluate the robustness of the method, a few critical system parameters, such as mobile composition, flow rate and pH of the mobile phase were deliberately varied and the resolution between closely eluting degradant and promethazine hydrochloride (Rs) was evaluated. Three different mobile phase compositions (acetonitrile: 25 mM K_2HPO_4 48 : 50, 50 : 50 and 52 : 48), flow rates (0.8, 1 and 1.2 mL · min^{-1}), pH's of the mobile phase (6.8, 7.0 and 7.2) were studied. Other responses measured include % RSD, the number of theoretical plates (N) and tailing factor (T). Six replicates of each sample were injected.

3.3.6. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products (ICH 2005). The specificity of the method was determined by the complete separation of PMZ in presence of its degradation products obtained by forced degradation studies (Kaul et al. 2005). Further, the specificity was also assessed by measuring the purity of the drug peak in all of the stressed sample solutions with a photodiode array detector (PDA).
Forced degradation studies were performed by subjecting the drug sample to acid, base and neutral hydrolysis, oxidation, reduction and dry heat. A stock solution having a concentration of 200 μg · mL^{-1} was prepared in water and was used for forced degradation.

3.3.6.1. Degradation by acid

For degradation of PMZ by acid, 1 mL of the stock solution (200 μg · mL^{-1}) was transferred to a 5-mL volumetric flask, and made up to volume with 1 N hydrochloric acid (HCl). The mixture was refluxed at 80 °C for 2 h, and then cooled to room temperature. The pH of the solution was adjusted to neutrality by adding 1 N sodium hydroxide (NaOH), filtered through a 0.45-μm syringe filter and injected into HPLC to detect peaks of degradation products.

3.3.6.2. Degradation by base

For degradation of PMZ by base, 1 mL of the stock solution of the stock solution (200 μg · mL^{-1}) was transferred to a 5-mL volumetric flask, and the volume made up with 1 N NaOH. The mixture was refluxed at 80 °C for 2 h, and then cooled to room temperature. The pH of the solution was adjusted to neutrality by adding 1 N HCl, filtered through a 0.45-μm syringe filter and injected into HPLC to detect peaks of degradation products.

3.3.6.3. Neutral hydrolysis

To 5 ml of stock solution (200 μg · mL^{-1}) in water were 5 mL of double distilled water added and the mixture was refluxed for 2 h at 80 °C. The solution was cooled to room temperature, filtered and injected into HPLC to detect degradation peaks of PMZ.

3.3.6.4. Degradation by reduction

The stock solution of PMZ (200 μg · mL^{-1}) was refluxed with 10 ml of 10% (w/v) sodium bisulfite solution ($NaHSO_3$) for 10 min to effect the degradation. The mixture was then cooled to room temperature, filtered using syringe filters and analyzed by HPLC.

3.3.6.5. Degradation by dry heat

The drug was subjected to dry heat at 80 °C for 3 days to effect partial degradation. The degraded sample was dissolved in methanol, filtered and analyzed by HPLC.

3.3.6.6. Degradation by oxidation

Stock solution (1 mL, 200 μg · mL^{-1}) was taken in a 5-mL volumetric flask and made up to volume with 3% (v/v) H_2O_2 solution. The mixture was refluxed for predetermined time intervals of 0, 5 and 15 min to evaluate the degradation of PMZ. At each time, samples were withdrawn, cooled to room temperature, filtered using 0.45-μm syringe filters and analyzed by HPLC for peaks of oxidation.

3.4. Solution stability

The stability of sample in solution is important to ensure that there is no degradation due to hydrolysis, photolysis or adhesion to glassware over the period of analysis. To assess the stability of the drug in the sample solution, the standard samples and controls were stored at room temperature for 10 days. The samples were then injected into the HPLC system and analyzed.

3.5. Analysis of promethazine hydrochloride in sustained release tablet formulation

A sustained release buccal tablet formulation of PMZ was prepared by a direct compression method. The prepared tablets were stored at 40 °C and 75% relative humidity (RH) and analyzed at pre-determined time intervals for up to 6 months via the HPLC method developed. To determine the PMZ remaining after storage at aforementioned conditions, each tablet (n = 20) was weighed individually and powdered. A quantity of powder equivalent to 10 mg of the drug was dissolved in 10 ml methanol in a volumetric flask, shaken on a vortex mixer for 2 min and centrifuged for 15 min at 4000 rpm. The clear supernatant obtained was diluted appropriately with methanol and injected into the liquid chromatographic system for analysis.

3.6. Analysis of promethazine hydrochloride in hot-melt extruded (HME) films

Polymeric film matrices incorporated with 20% PMZ were prepared by HME technology utilizing a single-screw Randcastle Microtruder (RCP-0250) at 90 °C. The films were stored at 40 °C and 75% RH and analyzed at pre-determined time intervals for up to 6 months to assess the stability of the drug in the films. For sample preparation, a known amount of the polymeric film was dissolved in methanol by sonicating for 10–15 min. The solution was filtered, transferred into vials and injected onto the HPLC column for analysis. The study was done in replicates of six.

3.7. Determination of drug content uniformity in HME films

Assessment of drug content uniformity in any dosage form developed is important as it can have a significant effect on its performance. Lack of content uniformity could lead to misinterpretation of results. Content uniformity was determined by sampling random portions of each film and analyzing for the amount of drug present utilizing the HPLC method developed.

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