

DRUG ASSAY HPLC METHODS AND CONDITIONS

1. METHOD TMD-013: IBUPROFEN 400 MG TABLET

1.1. EQUIPMENT AND REAGENTS

- 1.1.1. Ibuprofen Reference Standard
- 1.1.2. Chloroacetic Acid, ACS Grade or equivalent
- 1.1.3. Ammonium Hydroxide, ACS Grade or equivalent
- 1.1.4. Water, HPLC Grade or equivalent
- 1.1.5. Acetonitrile, HPLC Grade or equivalent
- 1.1.6. Methanol, HPLC Grade or equivalent
- 1.1.7. Class A Glassware
- 1.1.8. Syringe filter
- 1.1.9. Balance capable of weighing to ± 0.1 mg
- 1.1.10. HPLC System capable of injecting 10 μ L and equipped with a UV detector capable of operating at 254 nm.

1.2. SOLUTION PREPARATION

- 1.2.1. **Mobile Phase:** Dissolve 4.0 g of chloroacetic acid in 400 ml of water, adjust with ammonium hydroxide to a pH of 3.0 if necessary, add 600 ml of acetonitrile, and mix.
- 1.2.2. **Standard Solution:** 10.0 mg/ml of USP Ibuprofen RS in Mobile phase.
- 1.2.3. **Sample solution:** Nominally 10.0 mg/ml of ibuprofen prepared as follows.
Transfer NL T 10 Tablets to a suitable volumetric flask and add about 50% final volume of Mobile phase. Shake on a mechanical shaker for at least 60 min or until the Tablets are disintegrated. Dilute with Mobile phase to volume. Centrifuge a portion of the solution at about 3000 rpm for about 10 min or until a clear supernatant is obtained. Use the supernatant for analysis.

1.3. CHROMATOGRAPHIC CONDITIONS

- 1.3.1. **Column:** XBridge C18, 2.1-mm x 10-cm; 2.5- μ m or equivalent
- 1.3.2. **Flow Rate:** 1.0 mL/min
- 1.3.3. **Detector:** 254 nm
- 1.3.4. **Injection Volume:** 10 μ L

1.4. 7.4 SYSTEM SUITABILITY

- 1.4.1. **Sample:** Standard Solution
- 1.4.2. **Suitability Requirements:**
 - 1.4.2.1. Tailing Factor NMT 2.5
 - 1.4.2.2. The peak areas five consecutive injections of sample have a %RSD not more than (NMT) 2.0%

1.5. ANALYSIS

- 1.5.1. **Samples:** Standard solution and Sample solution
- 1.5.2. **Calculations:** Calculate the percentage of the labeled amount of ibuprofen (C₁₃H₁₈O₂) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of ibuprofen from the Sample solution

r_s = peak response of ibuprofen from the Standard solution

C_s = concentration of USP Ibuprofen RS in the Standard solution (mg/ml)

Cu= nominal concentration of ibuprofen in the Sample solution (mg/ml)

1.6. ACCEPTANCE CRITERIA: 90.0%-110.0%

2. METHOD TMD-016. ACETAMINOPHEN 500 MG TABLETS

2.1. EQUIPMENT AND REAGENTS

- 2.1.1. Acetaminophen Reference Standard
- 2.1.2. Glacial Acetic Acid, ACS Grade or equivalent
- 2.1.3. Water, HPLC Grade or equivalent
- 2.1.4. Methanol, HPLC Grade
- 2.1.5. Class A Glassware
- 2.1.6. Syringe filter
- 2.1.7. Balance capable of weighing to ± 0.1 mg
- 2.1.8. Gradient HPLC System capable of injecting 10 μ L, maintaining column temperature at 40 °C, and equipped with a UV detector capable of operating at 243 nm.

2.2. 7.2 SOLUTION PREPARATION

- 2.2.1. Solution A: 1 % v/v Glacial Acetic Acid in water.
- 2.2.2. Solution B: Methanol
- 2.2.3. Mobile Phase:

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
4.0	90	10
4.1	20	80
6.0	20	80
6.1	90	10
10.0	90	10

- 2.2.4. **Diluent:** Methanol:Water (10:90)
- 2.2.5. **Standard Solution:** 0.01 mg/ml of USP Acetaminophen RS in Diluent.
- 2.2.6. **Sample stock solution:** Nominally 0.1 mg/ml of Acetaminophen in Diluent prepared as follows. Transfer an appropriate amount of acetaminophen from NLT 10 Tablets to a suitable volumetric flask and dilute with Diluent to volume. Centrifuge or pass a portion of this solution through a suitable filter. [NoteSonication or shaking may be necessary.]
- 2.2.7. **Sample solution:** Nominally 0.01 mg/ml of acetaminophen in Diluent from the Sample stock solution. Pass a portion of this solution through a suitable filter.

2.3. CHROMATOGRAPHIC CONDITIONS

- 2.3.1. **Column:** XBridge C18, 3.0-mm x 10-cm; 3.5- μ m packing or equivalent
- 2.3.2. **Flow Rate:** 0.5 mL/min
- 2.3.3. **Detector:** 243 nm
- 2.3.4. **Injection Volume:** 10 μ L
- 2.3.5. **Column Temperature:** 40 °C

2.4. SYSTEM SUITABILITY

- 2.4.1. **Sample:** Standard Solution
- 2.4.2. **Suitability Requirements:**
 - 2.4.2.1. Tailing Factor NMT 2.0
- 2.4.3. The peak areas five consecutive injections of sample have a %RSD NMT 2.0%

2.5. ANALYSIS

- 2.5.1. **Samples:** Standard solution and Sample solution

2.5.2. **Calculations:** Calculate the percentage of the labeled amount of ibuprofen (C₈H₉O₂) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of ibuprofen from the Sample solution

r_s = peak response of ibuprofen from the Standard solution

C_s = concentration of USP Ibuprofen RS in the Standard solution (mg/ml)

C_u = nominal concentration of ibuprofen in the Sample solution (mg/ml)

2.6. ACCEPTANCE CRITERIA: 90.0%-110.0%

3. METHOD TMD-019 PROMETHAZINE HYDROCHLORIDE 25 MG TABLET

3.1. EQUIPMENT AND REAGENTS

- 3.1.1. Promethazine Hydrochloride Reference Standard
- 3.1.2. Promethazine Related Compound B Reference Standard
- 3.1.3. Hydrochloric Acid, ACS Grade or equivalent
- 3.1.4. Triethylamine, ACS Grade or equivalent
- 3.1.5. Water, HPLC Grade or equivalent
- 3.1.6. Acetonitrile, HPLC Grade or equivalent
- 3.1.7. Class A Glassware
- 3.1.8. Syringe filter, 0.45 µm
- 3.1.9. Balance capable of weighing to ± 0.1 mg
- 3.1.10. HPLC System capable of injecting 20 µL and equipped with a UV detector
- 3.1.11. capable of operating at 254 nm.

3.2. SOLUTION PREPARATION

- 3.2.1. **Diluent:** Dissolve 8.2 ml of Hydrochloric Acid in 1000 ml of water.
- 3.2.2. **Mobile Phase:** Acetonitrile, Water, and Triethylamine (850:270:1)
- 3.2.3. **System Suitability Stock Solution:** 1.2 mg/ml of USP Promethazine Related Compound B RS in Diluent. Sonicate to dissolve.
- 3.2.4. **Standard Solution:** 0.1 mg/ml of USP Promethazine Hydrochloride RS in Diluent. Sonicate to dissolve.
- 3.2.5. **System Suitability Solution:** 0.09 mg/ml of USP Promethazine Hydrochloride RS and 0.12 mg/ml of USP Promethazine Related Compound B RS in Diluent from the Standard solution and System suitability stock solution, respectively.
- 3.2.6. **Sample Stock Solution:** Nominally 2.5-5.0 mg/ml of Promethazine Hydrochloride prepared as follows. Transfer 20 Tablets to a volumetric flask of an appropriate size and add 50% of the flask volume of Diluent. Sonicate with swirling for NL T 20 min, or until the Tablets have fully disintegrated. Shake the flask for NL T 15 min and dilute with Diluent to volume.
- 3.2.7. **Sample solution:** Nominally 0.1 mg/ml of Promethazine Hydrochloride in Diluent from the Sample Stock Solution. Pass a portion through a filter of 0.45 µm pore size and use the clear filtrate.

3.3. CHROMATOGRAPHIC CONDITIONS

- 3.3.1. **Column:** XBridge C18, 3.9-mm x 30-cm, 10-µm or equivalent
- 3.3.2. **Flow Rate:** 2.5 ml/min
- 3.3.3. **Detector:** 254 nm
- 3.3.4. **Injection Volume:** 20 µl
- 3.3.5. **Run time:** NLT 2.5 times the retention time of Promethazine

3.4. SYSTEM SUITABILITY

3.4.1. **Samples:** System Suitability Solution and Standard Solution [NOTE-The relative retention times for promethazine related compound B and promethazine are 0.82 and 1.0, respectively.]

3.4.2. **Suitability Requirements:**

3.4.2.1. **Resolution:** NLT 1.5 between promethazine and promethazine related compound B, System Suitability Solution

3.4.2.2. Tailing Factor NMT 1.5

3.4.2.3. The peak areas five consecutive injections of Standard Solution have a %RSD NMT 2.0%

3.5. ANALYSIS

3.5.1. **Samples:** Standard solution and Sample solution

3.5.2. **Calculations:** Calculate the percentage of the labeled amount of ibuprofen (C₁₁H₂₀N₂S·HCl) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of ibuprofen from the Sample solution

r_s = peak response of ibuprofen from the Standard solution

C_s = concentration of USP Ibuprofen RS in the Standard solution (mg/ml)

C_u = nominal concentration of ibuprofen in the Sample solution (mg/ml)

3.6. ACCEPTANCE CRITERIA: 95.0%-110.0%

4. METHOD TMD-022 AMOXICILLIN 500 CAPSULES

4.1. EQUIPMENT AND REAGENTS

4.1.1. Amoxicillin Reference Standard

4.1.2. Monobasic Potassium Phosphate, ACS Grade or equivalent

4.1.3. 45% Potassium Hydroxide Test Solution

4.1.4. Water, HPLC Grade or equivalent

4.1.5. Acetonitrile, HPLC Grade

4.1.6. Class A Glassware

4.1.7. Syringe filter

4.1.8. Balance capable of weighing to ± 0.1 mg

4.1.9. HPLC System capable of injecting 10 μ L and equipped with a UV detector capable of operating at 230 nm.

4.2. SOLUTION PREPARATION

4.2.1. **Buffer:** Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1 .

4.2.2. **Mobile Phase:** Acetonitrile and Buffer (1 :24)

4.2.3. **Standard Solution:** 1.2 mg/ml of USP Amoxicillin RS in Buffer. [NOTE-Use this solution within 6 h.]

4.2.4. **Sample solution:** Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a quantity, equivalent to 200 mg of anhydrous amoxicillin, to a 200-ml volumetric flask. Add Buffer to volume. Sonicate if necessary to ensure complete dissolution. [NOTE-Use this solution within 6 h.]

4.3. CHROMATOGRAPHIC CONDITIONS

4.3.1. **Column:** XBridge C 18, 4-mm x 25-cm; 10- μ m packing or equivalent

- 4.3.2. **Flow Rate:** 1.5 mL/min
- 4.3.3. **Detector:** 230 nm
- 4.3.4. **Injection Volume:** 10 µL
- 4.4. SYSTEM SUITABILITY
 - 4.4.1. **Sample:** Standard Solution
 - 4.4.2. **Suitability Requirements:**
 - 4.4.2.1. Tailing Factor NMT 2.5
 - 4.4.2.2. The peak areas five consecutive injections of sample have a %RSD NMT 2.0%
- 4.5. ANALYSIS
 - 4.5.1. **Samples:** Standard solution and Sample solution
 - 4.5.2. **Calculations:** Calculate the percentage of the labeled amount of Amoxicillin (C₁₆H₁₉N₃O₅S) in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

 - r_u = peak response from the Sample solution
 - r_s = peak response from the Standard solution
 - C_s = concentration of USP Amoxicillin RS in the Standard solution (mg/ml)
 - C_u = nominal concentration of Amoxicillin in the Sample solution (mg/ml)
 - P = potency of Amoxicillin in USP Amoxicillin RS (µg/mg)
 - F = conversion factor, 0.001 mg/µg
- 4.6. ACCEPTANCE CRITERIA: 90.0%-120.0%

IMPURITY HPLC METHODS AND CONDITIONS

1. METHOD TMD-014: IBUPROFEN 400 MG TABLET

- 1.1. EQUIPMENT AND REAGENTS
 - 1.1.1. Ibuprofen Reference Standard
 - 1.1.2. Ibuprofen Related Compound C Reference Standard
 - 1.1.3. Ibuprofen Related Compound J Reference Standard
 - 1.1.4. Chloroacetic Acid, ACS Grade or equivalent
 - 1.1.5. Ammonium Hydroxide, ACS Grade or equivalent
 - 1.1.6. Water, HPLC Grade or equivalent
 - 1.1.7. Acetonitrile, HPLC Grade or equivalent
 - 1.1.8. Methanol, HPLC Grade or equivalent
 - 1.1.9. Class A Glassware
 - 1.1.10. Syringe filter
 - 1.1.11. Balance capable of weighing to ± 0.1 mg
 - 1.1.12. HPLC System capable of injecting 10 µL and equipped with a UV detector capable of operating at 254 nm.
- 1.2. SOLUTION PREPARATION
 - 1.2.1. **Mobile Phase:** Dissolve 4.0 g of chloroacetic acid in 400 ml of water, adjust with ammonium hydroxide to a pH of 3.0 if necessary, add 600 ml of acetonitrile, and mix.
 - 1.2.2. Sensitivity Solution: 0.005 mg/ml of USP Ibuprofen RS in Mobile phase

- 1.2.3. System Suitability Solution: 10.0 mg/ml of USP Ibuprofen RS and 0.01 mg/ml each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS in Mobile phase
- 1.2.4. Standard Solution: 0.02 mg/ml of USP Ibuprofen RS and 0.01 mg/ml each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS in Mobile phase
- 1.2.5. Sample Solution: Nominally 10.0 mg/ml of ibuprofen prepared as follows. Transfer NL T 10 Tablets to a suitable volumetric flask and add about 50% final volume of Mobile phase. Shake on a mechanical shaker for at least 60 min or until the Tablets are disintegrated. Dilute with Mobile phase to volume. Centrifuge a portion of the solution at about 3000 rpm for about 10 min or until a clear supernatant is obtained. Use the supernatant for analysis.

1.3. CHROMATOGRAPHIC CONDITIONS

- 1.3.1. Column: XBridge C18, 2.1-mm x 10-cm; 2.5- μ m or equivalent
- 1.3.2. Flow Rate: 1.0 ml/min
- 1.3.3. Detector: 254 nm
- 1.3.4. Injection Volume: 10 μ L

1.4. SYSTEM SUITABILITY

- 1.4.1. Samples: Sensitivity Solution, System Suitability Solution, and Standard Solution
- 1.4.2. Suitability Requirements:
 - 1.4.2.1. Resolution: NLT 2.5 between ibuprofen related compound J and ibuprofen; NLT 2.5 between ibuprofen and ibuprofen related compound C, System Suitability Solution
 - 1.4.2.2. Signal-to-noise ratio: NL T 10, Sensitivity Solution
 - 1.4.2.3. Relative Standard Deviation: NMT 6.0% for ibuprofen related compound J, ibuprofen, and ibuprofen related compound C, Standard solution

1.5. ANALYSIS

- 1.5.1. Samples: Standard Solution and Sample Solution
- 1.5.2. Calculations:
 - 1.5.2.1. Calculate the percentage of ibuprofen related compound J and ibuprofen related compound C in the portion of Tablets taken:

$$\text{Result} = (ru/rs) \times \{Cs/Cu\} \times 100$$

ru = peak response of ibuprofen related compound J or ibuprofen related compound C from the Sample solution

ru = peak response of any individual unspecified degradation product from the Sample solution

rs= peak response of ibuprofen from the Standard solution

Cs= concentration of USP Ibuprofen RS in the Standard solution (mg/ml)

Cu= nominal concentration of ibuprofen in the Sample solution (mg/ml)

- 1.6. ACCEPTANCE CRITERIA: See Table 1. Disregard any peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Ibuprofen related compound J	0.47	0.2
Ibuprofen	1.00	-
Ibuprofen related compound C	1.62	0.25
Any unspecified degradation product	-	0.2
Total degradation products	-	1.5

2. METHOD TMD-017: Acetaminophen Tablets, 500 mg

2.1. EQUIPMENT AND REAGENTS

- 2.1.1. Acetaminophen Reference Standard
- 2.1.2. 4-Aminophenol Reference Standard
- 2.1.3. Ammonium Formate, ACS Grade or equivalent
- 2.1.4. Formic Acid, ACS Grade or equivalent
- 2.1.5. Ammonium Acetate, ACS Grade or equivalent
- 2.1.6. Trifluoroacetic Acid, ACS Grade or equivalent
- 2.1.7. Water, HPLC Grade or equivalent
- 2.1.8. Acetonitrile, HPLC Grade
- 2.1.9. Methanol, HPLC Grade
- 2.1.10. Class A Glassware
- 2.1.11. Syringe filter
- 2.1.12. Balance capable of weighing to ± 0.1 mg
- 2.1.13. Gradient HPLC System capable of injecting 25 μ L, maintaining column temperature at 40 °C, and equipped with a UV detector capable of operating at 272 nm.

2.2. SOLUTION PREPARATION

- 2.2.1. Buffer: Dissolve 1.9 g of Ammonium Formate in 1 L of Water. Add 1.0 ml of Formic Acid.
- 2.2.2. Solution A: Dissolve 3.1 g of Ammonium Acetate in 1 L of Water. Add 1.0 ml of Trifluoroacetic Acid.
- 2.2.3. Solution 8 : Acetonitrile, Methanol, and Water (10:75:15)
- 2.2.4. Solution C: Dissolve 3.1 g of ammonium acetate in 1000 ml of Solution B. Add 1.0 ml of Trifluoroacetic acid.
- 2.2.5. Mobile Phase: See Table 1. Return to original conditions and re-equilibrate the system for 4 min.

Table 1

Time (min)	Solution A (%)	Solution C (%)
0	97	3
5	70	30
10	10	90
11	10	90
11.1	97	3
15	97	3

2.2.6. Diluent: Methanol and Buffer (5:95)

2.2.7. Sensitivity Solution: 0.000175 mg/ml of USP 4-Aminophenol RS in Diluent. Sonicate to dissolve, if necessary.

2.2.8. Standard Solution: 0.001 75 mg/ml of USP 4-Aminophenol RS and 0.0035 mg/ml of USP Acetaminophen RS in Diluent. Sonicate to dissolve, if necessary.

2.2.9. Sample Stock Solution: Nominally 5 mg/ml of Acetaminophen in Diluent from NLT 10 Tablets. [NOTE- It is recommended to shake on a flat bed at low speed (180 oscillations/min) to dissolve, if necessary.]

2.2.10. Sample solution: Nominally 3.5 mg/ml of Acetaminophen in Diluent prepared as follows. Pass a portion of the Sample stock solution through a suitable filter of 0.2- μ m pore size. Discard the first 2 ml of the filtrate. Dilute a suitable volume of the filtrate with Diluent to volume.

2.3. CHROMATOGRAPHIC CONDITIONS

2.3.1. Column: XBridge C18, 4.6-mm x 15-cm; 3- μ m packing or equivalent

2.3.2. Flow Rate: 0.9 mL/min

2.3.3. Detector: 272 nm

2.3.4. Injection Volume: 25 μ L

2.3.5. Column Temperature: 40 °C

2.4. SYSTEM SUITABILITY

2.4.1. Sample: Sensitivity Solution and Standard Solution

2.4.2. Relative Standard Deviation: NMT 5.0% for 4-Aminophenol and Acetaminophen, Standard solution

2.4.3. Signal-to-noise ratio: NLT 10 for 4-Aminophenol, Sensitivity solution

2.5. 7.5 ANALYSIS

2.5.1. Samples: Standard Solution and Sample Solution

2.5.2. Calculations:

2.5.2.1. Calculate the percentage of 4-aminophenol in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of 4-aminophenol from the Sample solution

r_s = peak response of 4-aminophenol from the Standard solution

C_s = concentration of USP 4-Aminophenol RS in the Standard solution (mg/ml)

C_u = nominal concentration of acetaminophen in the Sample solution (mg/ml)

2.5.2.2. Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of any unspecified impurity from the Sample solution

r_s = peak response of acetaminophen from the Standard solution

Cs = concentration of USP Acetaminophen RS in the Standard solution (mg/ml)

2.5.3. Cu = nominal concentration of acetaminophen in the Sample solution (mg/ml)

2.6. ACCEPTANCE CRITERIA: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
4-Aminophenol	0.53	0.15
Acetaminophen	1.0	-
Any unspecified impurity		0.15
Total impurities		0.60

3. METHOD TMD-017: Promethazine Hydrochloride 25 mg

3.1. EQUIPMENT AND REAGENTS

- 3.1.1. Promethazine Hydrochloride Reference Standard
- 3.1.2. Promethazine Related Compound B Reference Standard
- 3.1.3. Triethylamine, ACS Grade or equivalent
- 3.1.4. Water, HPLC Grade or equivalent
- 3.1.5. Ammonium Acetate, ACS Grade or equivalent
- 3.1.6. Methanol, HPLC Grade or equivalent
- 3.1.7. Acetonitrile, HPLC Grade or equivalent
- 3.1.8. Class A Glassware
- 3.1.9. Syringe filter, 0.45 µm
- 3.1.10. Balance capable of weighing to ± 0.1 mg
- 3.1.11. Gradient HPLC System capable of injecting 15 µL and equipped with a UV detector capable of operating at 234 and 249 nm.

3.2. SOLUTION PREPARATION

- 3.2.1. **Diluent:** Methanol and Triethylamine (999:1).
- 3.2.2. **Buffer:** 3.7 g/L of Ammonium Acetate in water.
- 3.2.3. **Solution A:** Buffer and Acetonitrile (700:300)
- 3.2.4. **Solution B:** Acetonitrile
- 3.2.5. **Mobile Phase:**

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

- 3.2.6. **System Suitability Stock Solution:** 0.5 mg/ml of USP Promethazine Related Compound B RS in Diluent.
- 3.2.7. **Standard Stock Solution:** 0.5 mg/ml of USP Promethazine Hydrochloride RS in Diluent.
- 3.2.8. **System Suitability Solution:** 5 µg/ml each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS from the Standard Stock solution and System Suitability Stock Solution, respectively
- 3.2.9. **Standard Solution:** 5 µg/ml of USP Promethazine Hydrochloride RS from the Standard Stock Solution
- 3.2.10. **Sensitivity Solution:** 0.25 µg/ml of USP Promethazine Hydrochloride RS from the Standard Solution
- 3.2.11. **Sample Solution:** Nominally 0.5 mg/ml of promethazine hydrochloride from powdered Tablets (NL T 20) prepared as follows. Transfer a quantity of powdered Tablets, equivalent to 50 mg of promethazine hydrochloride, to a volumetric flask of appropriate size and add 75% of the flask volume of Diluent. Shake the flask for NL T 5 min and dilute with Diluent to volume. Pass a portion through a suitable filter.

3.3. CHROMATOGRAPHIC CONDITIONS

- 3.3.1. Column: XBridge C184.6-mm x 15-cm; 5-µm or equivalent
- 3.3.2. Column Temperature: 30°C
- 3.3.3. Flow Rate: 1.4 ml/min
- 3.3.4. Detector: 234 and 249 nm
- 3.3.5. Injection Volume: 15 µL

3.4. SYSTEM SUITABILITY

- 3.4.1. **Samples:** System Suitability Solution, Standard Solution, and Sensitivity Solution [NOTE-See Table 2 for the relative retention times.]

3.4.2. Suitability Requirements:

- 3.4.2.1. **Resolution:** NLT 5.0 between Promethazine and Promethazine Related Compound B, System Suitability Solution
- 3.4.2.2. **Relative Standard Deviation:** NMT 3.0% at 234 and 249 nm, Standard Solution
- 3.4.2.3. **Signal-to-noise ratio:** NLT 10 at 234 and 249 nm, Sensitivity solution

3.5. ANALYSIS

- 3.5.1. **Samples:** Standard Solution and Sample Solution

3.5.2. Calculations:

- 3.5.2.1. Calculate the percentage of Promethazine Sulfoxide in the portion of Tablets taken:

$$\text{Result} = (ru/rs) \times (Cs/Cu) \times (1/F) \times 100$$

ru = peak response of promethazine sulfoxide at 234 nm from the Sample Solution
rs= peak response of promethazine hydrochloride at 234 nm from the Standard solution

Cs= concentration of USP Promethazine Hydrochloride RS in the Standard Solution (mg/ml)

Cu = nominal concentration of promethazine hydrochloride in the SampleSolution (mg/ml)

F =relative response factor (see Table 2)

3.5.2.2. Calculate the percentage of all other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each degradation product at 249 nm from the sample solution

r_s = peak response of promethazine hydrochloride at 249 nm from the standard solution

C_s = concentration of USP Promethazine Hydrochloride RS in the standard solution (mg/ml)

C_u = nominal concentration of promethazine hydrochloride in the Sample solution (mg/ml)

3.6. ACCEPTANCE CRITERIA: See Table 2. Disregard peaks that are less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Promethazine sulfoxide ^a	0.28	2.1	0.5
Desmethyl promethazine ^b	0.71	1.0	0.5
Promethazine	1.0	-	-
Promethazine related compound B ^c	1.3	-	-
Phenothiazine	1.7	2.0	0.5
Any individual unspecified degradation product	-	1.0	0.2
Total degradation products	-	-	1.0

^a *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.

^b *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

^c This is a process impurity and is included for identification only. It is not to be reported and not to be included in the total degradation products.

4. Method TDM-023 Amoxicillin Capsules, 500 mg

4.1. EQUIPMENT AND REAGENTS

4.1.1. Amoxicillin Reference Standard

4.1.2. Amoxicillin Related Compound C Reference Standard

4.1.3. Amoxicillin Related Compound H Reference Standard

4.1.4. Monobasic Potassium Phosphate, ACS Grade or equivalent

4.1.5. 20% Sodium Hydroxide Solution

- 4.1.6. Water, HPLC Grade or equivalent
- 4.1.7. Acetonitrile, HPLC Grade
- 4.1.8. Class A Glassware
- 4.1.9. Syringe filter
- 4.1.10. Balance capable of weighing to ± 0.1 mg
- 4.1.11. HPLC System capable of injecting 10 μ L and equipped with a UV detector capable of operating at 230 nm.

4.2. SOLUTION PREPARATION

- 4.2.1. **Solution A:** Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 20% (w/v) solution of sodium hydroxide to a pH of 5.0 ± 0.1 .
- 4.2.2. **Solution B:** Acetonitrile
- 4.2.3. **Mobile Phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
25	94	6
40	84	16
50	84	16
51	100	0
60	100	0

- 4.2.4. Impurity Stock Solution: 0.15 mg/ml each of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS in Solution A, prepared as follows. Transfer a weighed amount of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS to a suitable volumetric flask. Add acetonitrile to fill 10% of the flask volume and Solution A to fill 60% of the flask volume. Sonicate to dissolve and dilute with Solution A to volume.
 - 4.2.5. System Suitability Solution: 1.5 mg/ml of USP Amoxicillin RS and 0.015 mg/ml each of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS in Solution A prepared as follows. Transfer a weighed amount of USP Amoxicillin RS to a suitable volumetric flask. Add Solution A to fill 60% of the flask volume. Add an appropriate volume of Impurity stock solution to the volumetric flask. Sonicate to dissolve and dilute with Solution A to volume.
 - 4.2.6. Standard Solution: 0.017 mg/ml of USP Amoxicillin RS in Solution A. Sonicate if necessary to dissolve. Use this solution immediately after preparation.
 - 4.2.7. Sample solution: Nominally 1.5 mg/ml of amoxicillin in Solution A from the Capsules, prepared as follows. Transfer Capsule powder equivalent to 75 mg of amoxicillin into a 50-ml volumetric flask. Add Solution A to fill 60% of the final flask volume. Sonicate for 15 min and dilute with Solution A to volume. Pass through a suitable filter of 0.45- μ m pore size. Use this solution immediately after preparation.
- #### 4.3. 7.3 CHROMATOGRAPHIC CONDITIONS
- 4.3.1. Column: XBridge C8, 4.6-mm x 15-cm; 5- μ m packing packing or equivalent

4.3.2. Column Temperature: 40 °C

4.3.3. Flow Rate: 2 ml/min

4.3.4. Detector: 230 nm

4.3.5. Injection Volume: 20 µL

4.4. SYSTEM SUITABILITY

4.4.1. Sample: System Suitability Solution and Standard Solution. [NOTE-See Table 2 for relative retention times.]

4.4.2. Suitability Requirements:

4.4.3. Resolution: NL T 1.5 between amoxicillin related compound C and amoxicillin related compound H, System suitability solution

4.4.4. Relative Standard Deviation: NMT 5.0%, Standard Solution.

4.5. 7.5 ANALYSIS

4.5.1. Samples: Standard solution and Sample solution

4.5.2. Calculations:

4.5.3. Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (ru/rs) \times (Cs/Cu) \times Px (F1/F2) \times 100$$

ru = peak response of each degradation product from the Sample solution

rs = peak response of amoxicillin from the Standard solution

Cs = concentration of USP Amoxicillin RS in the Standard solution (mg/ml)

Cu = nominal concentration of amoxicillin in the Sample solution (mg/ml)

P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)

F1 = conversion factor, 0.001 mg/µg

F2 = relative response factor (see Table 2)

4.6. ACCEPTANCE CRITERIA: See Table 2. Disregard any peak less than 0.05%.S