Anagrelide Hydrochloride

\[
\text{C}_{10}\text{H}_7\text{Cl}_2\text{N}_3\text{O} \cdot \text{HCl} \cdot \text{H}_2\text{O} \quad 310.56
\]
Anhydrous \quad 292.55

[58579-51-4].

Imidazo[2,1-b]quinoxalinol-2(3H)-one, 6,7-dichloro-1,5-
dihydro-, monohydrochloride, monohydrate; 6,7-Dichloro-1,5-dihydroimidazo[2,1-b]-quinoxalinol-2(3H)-
one monohydrochloride, monohydrate [823178-43-4].

DEFINITION
Anagrelide Hydrochloride contains NLT 98.0% and NMT 102.0% of anagrelide hydrochloride \((\text{C}_{10}\text{H}_7\text{Cl}_2\text{N}_3\text{O} \cdot \text{HCl})\), calculated on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.
- **C. IDENTIFICATION TESTS---GENERAL, Chloride (191):** Meets the requirements

ASSAY
- **PROCEDURE** Use freshly prepared standard and sample solutions and inject within 2 h.
  - **Solution A:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
  - **Mobile phase:** Acetonitrile and Solution A (1:3)
  - **Diluent:** Acetonitrile and water (1:1)
  - **Standard stock solution:** 0.5 mg/mL of anagrelide hydrochloride in acetonitrile prepared as follows. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add a small amount of 2 N hydrochloric acid (3 drops per every 50 mL of the final volume) and acetonitrile equivalent to fill about 80% of the final volume. Sonicate to dissolve, and dilute with acetonitrile to volume.
  - **Standard solution:** 0.05 mg/mL of anagrelide hydrochloride in Diluent from Standard stock solution
  - **Sample stock solution:** Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask, add 3 drops of 2 N hydrochloric acid and 40 mL of acetonitrile. Sonicate to dissolve, and dilute with acetonitrile to volume.
  - **Sample solution:** Transfer 5 mL of Sample stock solution to a 50-mL volumetric flask, and dilute with Diluent to volume.

Chromatographic system
(See Chromatography (621), System Suitability.)

Mode: LC
Detector: UV 254 nm
Column: 4.6-mm x 15-cm; 4-μm packing L11
Flow rate: 1.2 mL/min
Injection volume: 20 μL
System suitability
- **Sample:** Standard solution
- **Suitability requirements**
  - **Column efficiency:** NMT 3000 theoretical plates
  - **Tailing factor:** NMT 2.0
  - **Relative standard deviation:** NMT 2.0%

Analysis
- **Samples:** Standard solution and Sample solution
Calculate the percentage of anagrelide hydrochloride \((\text{C}_{10}\text{H}_7\text{Cl}_2\text{N}_3\text{O} \cdot \text{HCl})\) in the portion of Anagrelide Hydrochloride taken:

\[
\text{Result} = \left(\frac{r_U}{r_S}\right) \times \left(\frac{C_U}{C_S}\right) \times 100
\]

- \(r_U\) = peak response of anagrelide from the Sample solution
- \(r_S\) = peak response of anagrelide from the Standard solution
- \(C_U\) = concentration of USP Anagrelide Hydrochloride RS in the Standard solution (mg/mL)
- \(C_S\) = concentration of Anagrelide Hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 98.0%-102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS, Method II (231):** NMT 20 ppm

Change to read:

- **ORGANIC IMPURITIES**
  Use freshly prepared standard and sample solutions and inject within 2 h.
  - **Mobile phase:** Proceed as directed in the Assay.
  - **Diluent A:** Use the Diluent as described in the Assay.
  - **Diluent B:** Acetonitrile and water (1:3)
  - **Standard stock solution A:** 0.05 mg/mL of USP Anagrelide Related Compound A RS in Diluent A
  - **Standard stock solution B:** 0.05 mg/mL of anagrelide related compound B in acetonitrile.
  - **Standard stock solution C:** 0.1 mg/mL of anagrelide hydrochloride in acetonitrile.
  - **Standard stock solution D:** 0.25 μg/mL of each of anagrelide related compound A and anagrelide related compound B in Mobile phase from Standard stock solution A and Standard stock solution B
  - **Standard solution:** 0.05 μg/mL of anagrelide hydrochloride in Mobile phase from Standard stock solution C
  - **Sample stock solution:** Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask. Add 45 mL of acetonitrile, sonicate, and swirl the flask until the preparation turns into a cloudy liquid. Add 1 drop of 0.12 N hydrochloric acid (3 drops per every 50 mL of the final volume) and acetonitrile equivalent to fill about 80% of the final volume. Sonicate to dissolve, heat in the hot water bath if necessary, and dilute with acetonitrile to volume.

System suitability solution: 0.25 μg/mL of each of anagrelide related compound A and anagrelide related compound B in Mobile phase from Standard stock solution C and Standard stock solution B

Sample solution: Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask. Add 45 mL of acetonitrile, sonicate, and swirl the flask until the preparation turns into a cloudy liquid. Add 1 drop of 0.12 N hydrochloric acid (3 drops per every 50 mL of the final volume) and acetonitrile equivalent to fill about 80% of the final volume. Sonicate to dissolve, heat in the hot water bath if necessary, and dilute with acetonitrile to volume.
acid, swirl the flask until the liquid turns to clear, and dilute with acetonitrile to volume.

**Sample solution:** Transfer 5 mL of Sample stock solution into a 50-mL volumetric flask, and dilute with Diluent B to volume.

**Chromatographic system**
(See Chromatography (621), System Suitability.)

Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 4-µm packing L11
Auto sampler temperature: 5°
Flow rate: 1.2 mL/min
Injection volume: 50 µL

**System suitability**

Samples: System suitability solution and Standard solution

<table>
<thead>
<tr>
<th>Relative</th>
<th>Relative</th>
<th>Acceptance</th>
<th>Criteria,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention</td>
<td>Response</td>
<td>, NMT (%)</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anagrelide open ring methyl ester (if present)</td>
<td>0.80</td>
<td>0.51</td>
<td>0.25  (RB 1-Jun-2013)</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>1.00</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Anagrelide related compound C</td>
<td>1.41</td>
<td>0.32</td>
<td>0.15</td>
</tr>
<tr>
<td>Anagrelide trichloro derivative</td>
<td>2.44</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Any unspecified impurity</td>
<td>—</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
</tr>
</tbody>
</table>

- (2-Amino-5,6-dichloroquinazolin-3(4H)-yl)acetic acid.
- Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.
- Methyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4H)-yl)acetate.
- Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4H)-yl)acetate hydrobromide.
- 6,7,8-Trichloro-3,5-dihydropyridazino[2,1-b]quinazolin-2(1H)-one.

**SPECIFIC TESTS**

- **Water Determination, Method I (921):** 4.5%–7.5%

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store in a cold place.

- **USP Reference Standards (11)**
  - USP Anagrelide Hydrochloride RS
  - Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.
  - C₂₁H₁₂Cl₂N₂O₂ 277.15
  - USP Anagrelide Related Compound B RS
  - (2-Amino-5,6-dichloroquinazolin-3(4H)-yl)acetic acid.
  - C₁₀H₉Cl₂N₃O₂ 274.10

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**Table 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
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<tbody>
<tr>
<td>Anagrelide related compound A</td>
<td>0.55</td>
<td>0.37</td>
<td>0.15</td>
</tr>
<tr>
<td>Anagrelide related compound A²</td>
<td>0.40</td>
<td>0.43</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- (2-Amino-5,6-dichloroquinazolin-3(4H)-yl)acetic acid.
- Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.
- Methyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4H)-yl)acetate.
- Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4H)-yl)acetate hydrobromide.
- 6,7,8-Trichloro-3,5-dihydropyridazino[2,1-b]quinazolin-2(1H)-one.

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