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⟨227⟩ 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS

INTRODUCTION

This chapter provides a procedure and acceptance criterion (limit) to control the principal degradation product of acetaminophen, 4-aminophenol, an impurity that can form by hydrolysis of acetaminophen.

SOLUTION PREPARATIONS

All solution preparations that contain acetaminophen or 4-aminophenol should be protected from light and should be stored only for as long as can be supported by solution stability data acquired during verification under actual conditions of use.

Buffer: 4.0 g/L of sodium citrate dihydrate and 1.5 g/L of anhydrous citric acid, in water

Diluent: Acetonitrile and *Buffer* (10:90)

Solution A: 10 mM phosphate buffer prepared as follows. Add 0.60 g of monobasic potassium phosphate and 0.82 g of anhydrous dibasic sodium phosphate to a 1-L volumetric flask. Dissolve and dilute with water to volume to a pH of 7.0.

Solution B: Water

Solution C: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	90	5	5
5	90	5	5
7	10	10	80
7.1	90	5	5
10	90	5	5

Standard stock solution: 25 µg/mL of USP 4-Aminophenol RS in *Diluent*. Prepare fresh in conjunction with the other solution preparations described below. Discard after 4 h or as supported by solution stability data. [Note—See *Chromatographic Adjustments*, solution stability, below.]

System suitability solution: 2.5 µg/mL of USP 4-Aminophenol RS in *Diluent*, from the *Standard stock solution*

Sample stock solution: Nominally 10 mg/mL of acetaminophen from a suitable quantity of drug product in *Diluent*. [Note—Either component of the *Diluent* may be introduced to the drug product first, followed by addition of the other component to maintain the proportions of acetonitrile and *Buffer* and to achieve the appropriate final volume defined for the *Diluent*.]

[Note—It is recommended that the *Sample solution* and *Standard solution* be prepared concurrently within a narrow window of time (e.g., 30 min) for each drug product sample.]

Standard solution: Add 25.0 mL of the *Sample stock solution* and 15.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

Sample solution: Add 25.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

CHROMATOGRAPHIC METHOD

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 300 nm

Column: 4.6-mm × 15-cm; 5-µm packing L85

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The typical retention time for 4-aminophenol is about 4.2–5.3 min.]

Suitability requirements

Resolution: NLT 1.0 between 4-aminophenol and the nearest peak, *Standard solution*

Tailing factor: NMT 1.5 for the 4-aminophenol peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 20 for the 4-aminophenol peak, *System suitability solution*

ANALYSIS

Samples: *Standard solution* and *Sample solution*

Inject the *Sample solution* and *Standard solution* for each drug product sample sequentially, i.e., back-to-back.

Calculate the percentage of 4-aminophenol (C_6H_7NO) relative to acetaminophen in the portion of drug product taken:

$$\text{Result} = [r_U/(r_S - r_U)] \times (W_S/W_U) \times 100$$

r_U = peak response of 4-aminophenol from the *Sample solution*

r_S = peak response of 4-aminophenol from the *Standard solution*

W_S = amount of USP 4-Aminophenol RS added to the *Standard solution* (mg)

W_U = amount of acetaminophen in the *Sample solution* (mg)

Acceptance criteria (unless otherwise stated in the monograph)**:** NMT 0.15% of 4-aminophenol relative to acetaminophen

CHROMATOGRAPHIC ADJUSTMENTS

The retention time of 4-aminophenol can be tuned to achieve specificity for a given product matrix. This allowance supersedes provisions in ⟨621⟩ for adjusting chromatographic conditions and is intended to provide a measure of flexibility when needed. Suggestions for changing 4-aminophenol retention are given in *Table 2*. The use of a ternary mobile phase system affords ready changes to the ionic strength (water from *Solution B*) and organic strength (acetonitrile from *Solution C*), but this can be simplified to a binary mobile phase system.

Table 2

Condition Change	Change in 4-Aminophenol Retention
Increase in organic strength (*Solution C*)	Decreases 4-aminophenol retention
Decrease in pH (*Solution A*)	Increases 4-aminophenol retention
Increase in ionic strength (*Solution B*)	Decreases 4-aminophenol retention
Increase in column temperature	Increases 4-aminophenol retention

Adjustments to the chromatographic procedure may require verification or validation. See *Validation of Compendial Procedures* ⟨1225⟩ and *Verification of Compendial Procedures* ⟨1226⟩ for guidance. Adjusted chromatographic conditions must meet all system suitability requirements.

Solution stability must be verified under actual conditions of use to ensure 4-aminophenol is stable in the *Sample solution* and the *Standard solution* as evidenced by NMT ±10% change of the 4-aminophenol peak areas.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 4-Aminophenol RS

4-Amino-1-hydroxybenzene.

C_6H_7NO 109.13

Topic/Question	Contact	Expert Committee
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