Application Note

Small Molecule Pharmaceuticals

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Hyoscine Butylbromide Analysis by British Pharmacopoeia Method and 1260 Infinity II Prime LC

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Abstract

This Application Note illustrates the use of the Agilent 1260 Infinity II Prime LC for the analysis of hyoscine butylbromide and specified impurities as described by British Pharmacopoeia method 2.2.29. The 1260 Infinity II Prime LC is ideal for this analysis due to the extended power range of 800 bar, which is a key requirement for this method, where the highest method pressure goes up to 650 bar. An Agilent ZORBAX StableBond Column SBAQ delivered robust results with faster equilibration. The 1260 Infinity II flexible pump provided precise solvent delivery at a maximum flow rate of 5 mL/min up to 800 bar (~11,700 psi).

Introduction

Hyoscine butylbromide, also known as scopolamine butylbromide and sold under the brand name Buscopan among others, is a medication used to treat abdominal cramps, esophageal spasms, renal colic, and bladder spasms. Hyoscine butylbromide can be taken by mouth or by injection into a muscle or vein.

The British Pharmacopoeia has published a related substance (RS) method effective on January 1, 2019, which describes the use of a sub-2 µm, 4.6 mm id column for RS analysis. There are two impurities, referred to as impurity A and impurity B, which are tested along with hyoscine butylbromide as specified by the British Pharmacopoeia method.

Experimental

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Multisampler (G7167A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C)

Software

Agilent OpenLab CDS Workstation Version 2.3.0 (M8413AA)

Chromatographic conditions

Parameter	Value
Column	Agilent ZORBAX SBAQ, 4.6 mm × 10 cm, 1.8 µm packing
Mobile Phase	A) Acetonitrile R1, 0.2% v/v solution of perchloric acid R (5:95 v/v) B) 0.2% v/v solution of perchloric acid R, acetonitrile R1 (30:70 v/v)
Gradient	Time (min) / Mobile phase A (% v/v) / Mobile phase B (% v/v) 0–1 / 91 / 9 1–4.2 / 91–75 / 9–25 4.2–5.5 / 75–66 / 25–34 5.5–10 / 66–15 / 34–85 10–11 / 15 / 85
Flow Rate	2.5 mL/min
Injection Volume	2 µL
Column Temperature	50 °C
Detection	210 nm
Run Time	15 minutes

Reference solution preparation

- **Test solution:** 50.0 mg of the active pharma ingredient (API) to be examined was dissolved in mobile phase B and diluted to 10.0 mL with mobile phase B.
- **Reference solution A:** 1.0 mL of the test solution was diluted to 100.0 mL with mobile phase B, then 1.0 mL of this solution was diluted to 10.0 mL with mobile phase B.
- **Reference solution B:** Five milligrams of hyoscine butylbromide chemical reference substance (CRS, containing impurities A and B) for system suitability was dissolved in mobile phase B and diluted to 10.0 mL with mobile phase B.

Results and discussion

Mobile phase B was used as a blank, followed by injection of reference solution A and reference solution B for identification of the retention times (RTs) of impurity A, impurity B, and hyoscine butylbromide.

The precision of the method was tested by injecting reference solution B (Figure 4). The maximum backpressure observed for the method was 680 bar (Figure 5).

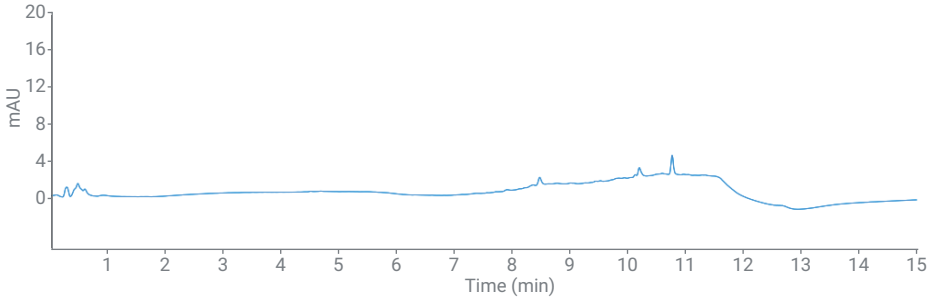

Figure 1. Blank.

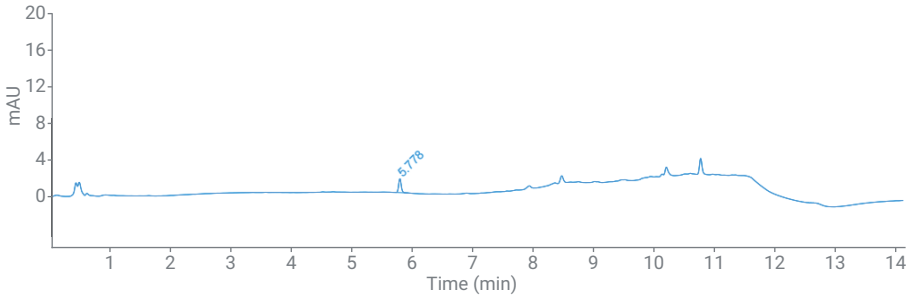

Figure 2. Reference solution A.

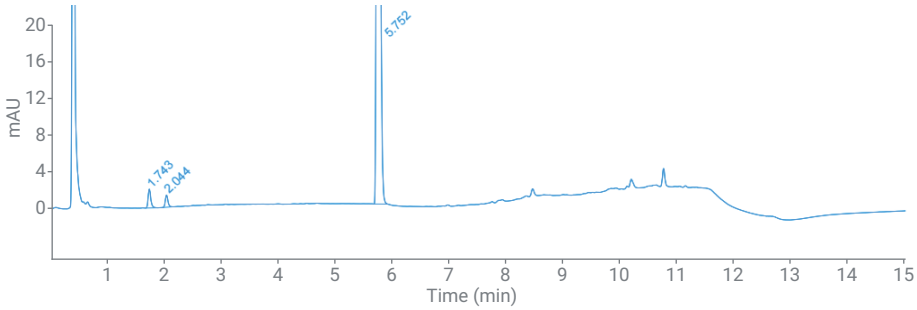

Figure 3. Reference solution B.

No.	SST Criteria	Expected	Observed	Pass/Fail
1	Minimum 2.0 between the peaks due to impurities B and A	2	3.6	Pass

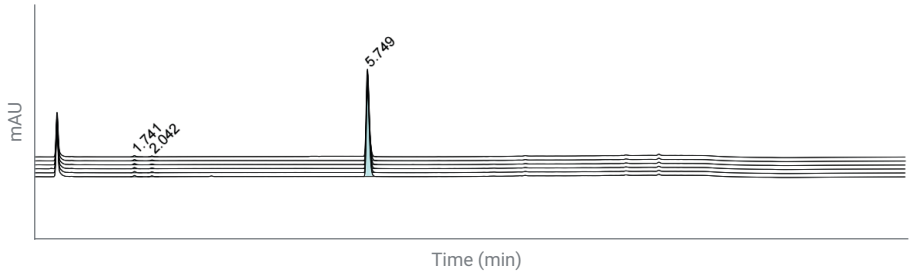

Figure 4. Overlay of six replicate injections of reference solution B.

Hyoscine						
Name	Inj.	Vial	RT	Area	Peak Area%	Height
SST	1	2	5.75	506	97.88	143
SST	2	2	5.75	508	97.89	143
SST	3	2	5.75	506	97.88	142
SST	4	2	5.75	505	97.90	142
SST	5	2	5.75	505	97.89	141
SST	6	2	5.75	505	97.88	141
		%RSD	0.02	0.20		1
		Mean	5.75	505.76		142
		Std. dev.	0.00	1.03		1

Impurity A						
Name	Inj.	Vial	RT	Area	Peak Area%	Height
SST	1	2	2.04	4	0.83	1
SST	2	2	2.04	4	0.83	1
SST	3	2	2.04	4	0.84	1
SST	4	2	2.04	4	0.84	1
SST	5	2	2.04	4	0.84	1
SST	6	2	2.04	4	0.84	1
		%RSD	0.08	0.49		0
		Mean	2.04	4.32		1
		Std. dev.	0.00	0.02		0

Impurity B						
Name	Inj.	Vial	RT	Area	Peak Area%	Height
SST	1	2	1.74	7	1.29	2
SST	2	2	1.74	7	1.29	2
SST	3	2	1.74	7	1.28	2
SST	4	2	1.74	7	1.27	2
SST	5	2	1.74	7	1.27	2
SST	6	2	1.74	7	1.29	2
		%RSD	0.06	0.78		1
		Mean	1.74	6.61		2
		Std. dev.	0.00	0.05		0

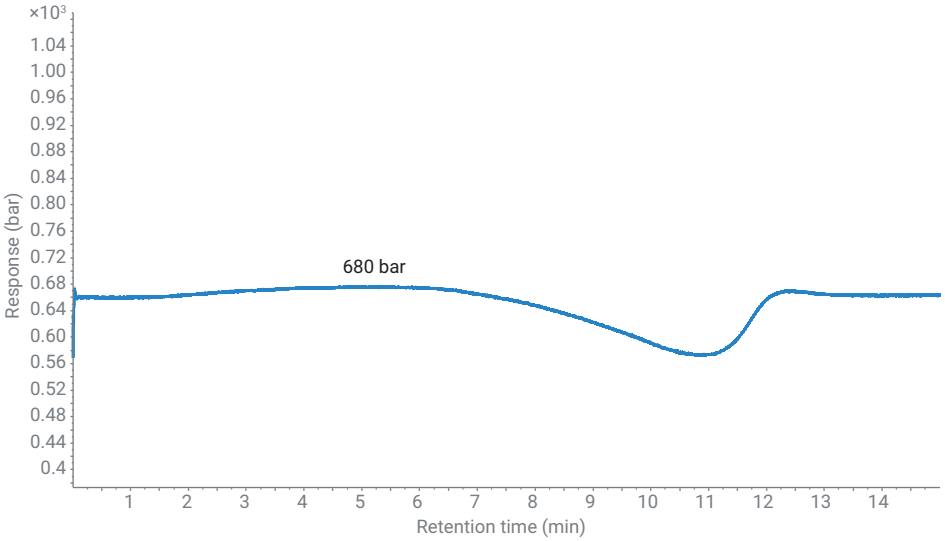

Figure 5. Pressure plot.

Conclusion

The Agilent 1260 Infinity II Prime LC, which uses a sub-2 µm column, is well suited for the analysis of hyoscine butylbromide according to the British Pharmacopoeia method. Use of this column necessitates using an UHPLC system; the Agilent 1260 Infinity II Prime LC is an excellent instrument for this analysis, as the method backpressure is <700 bar. Precision of <0.1% RSD of RT was observed for all peaks, demonstrating the reproducibility of the method.

Reference

1. Hyoscine Butylbromide. *British Pharmacopoeia* (2.2.29).

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