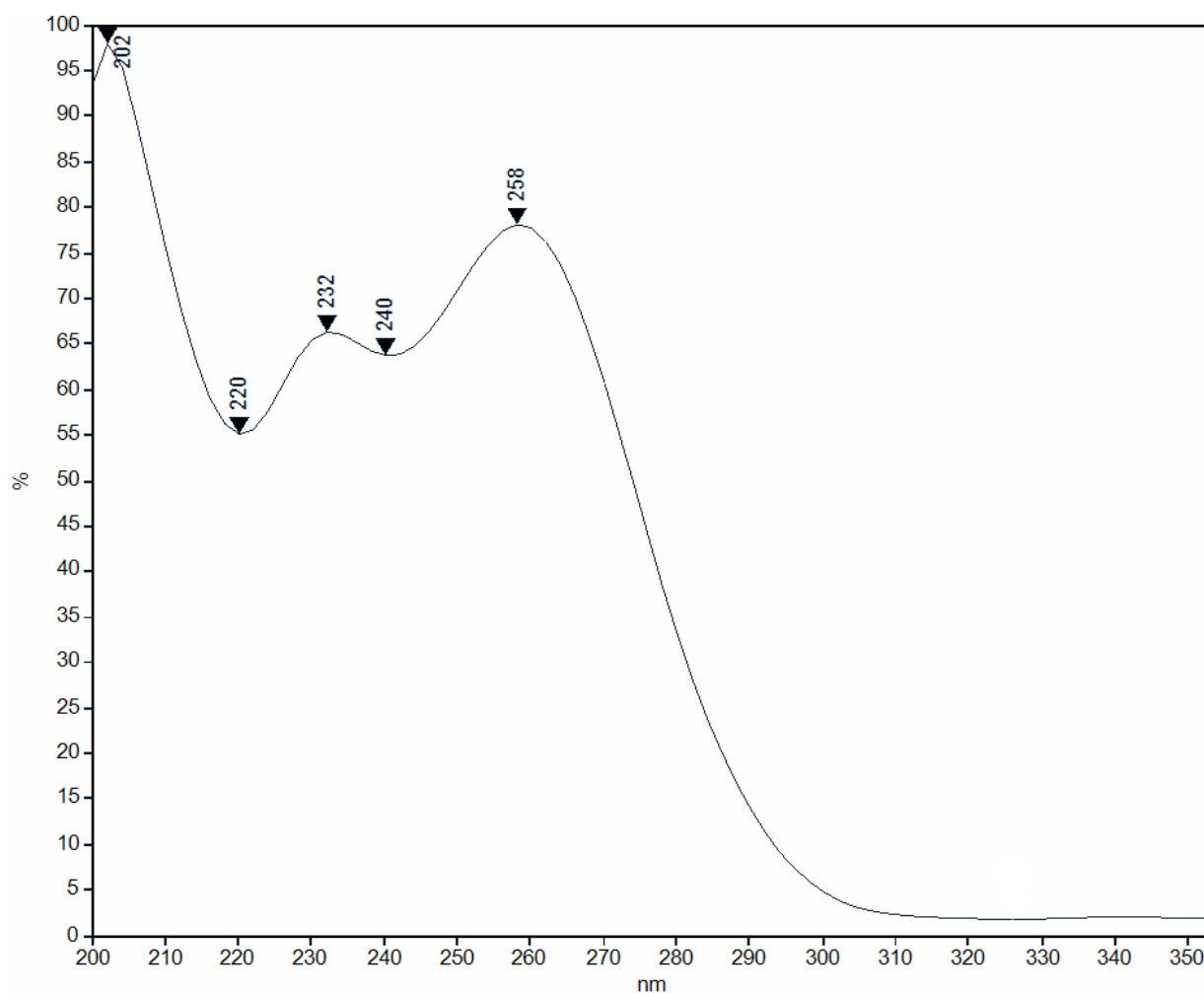


Chlorhexidine Mouthwash – BP 2024

These chromatograms are provided for information only as an aid to analysts and are intended as guidance for the interpretation and application of BP monographs.

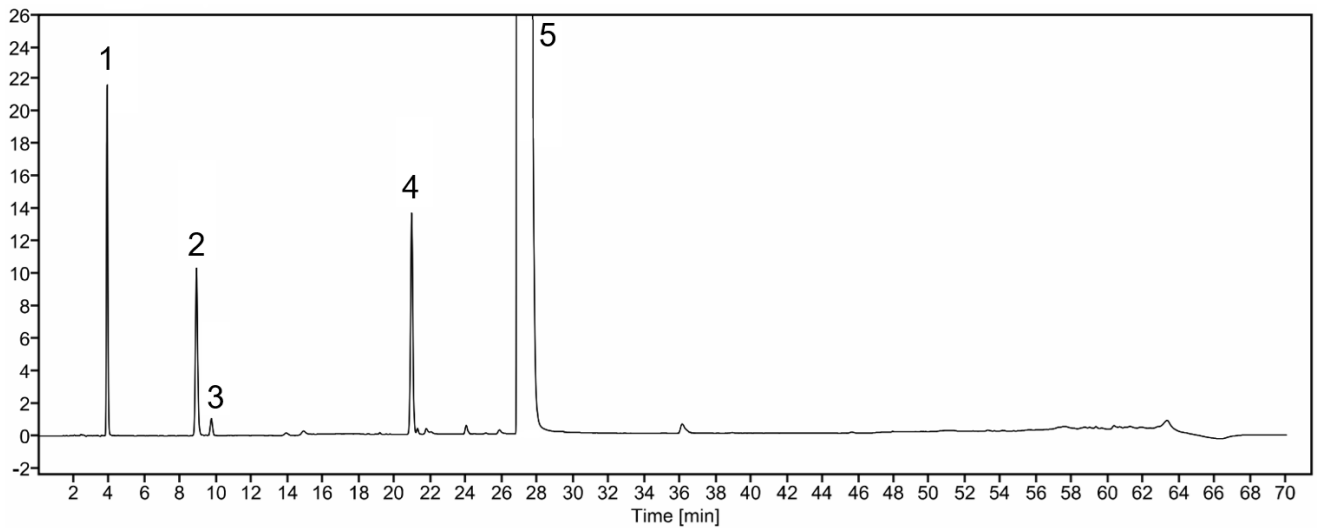
Typical spectrum for the Identification by HPLC-UV/DAD test for Chlorhexidine Mouthwash as published in BP 2024.



Column	Dr. Maisch, ReproSil-Pur Basic C18 (250 mm x 4.6 mm, 5 µm)			
Method Ref.	Assay for the Chlorhexidine Mouthwash monograph from BP 2024			
Buffer	10% v/v Trifluoroacetic acid in acetonitrile			
Mobile Phase A	Buffer: acetonitrile: water (1.8:180:830, v/v)			
Mobile Phase B	Buffer: water: acetonitrile (0.2:200:800, v/v/v)			
Diluent	Mobile phase A			
Flow rate	1.0 mL/min			
Column Temp	40 °C			
Injection Volume	25 µL			
Detection	250 nm			
Gradient	Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
	0 – 8	100	0	isocratic
	8 – 12	100 → 87	0 → 13	linear gradient
	12 – 20	87 → 86	13 → 14	linear gradient
	20 – 22	86	14	isocratic
	22 – 30	86 → 81	14 → 19	linear gradient
	30 – 40	81 → 60	19 → 40	linear gradient
	40 – 54	60 → 0	40 → 100	linear gradient
	54 – 60	0	100	isocratic
	60 – 62	0 → 100	100 → 0	linear gradient
	62 – 70	100	0	re-equilibration

These chromatograms are provided for information only as an aid to analysts and are intended as guidance for the interpretation and application of BP monographs.

Example chromatogram for Solution (4) during method development (without impurity H) illustrating SST requirements in the Related Substances test for Chlorhexidine Dental Gel as published in BP 2024.

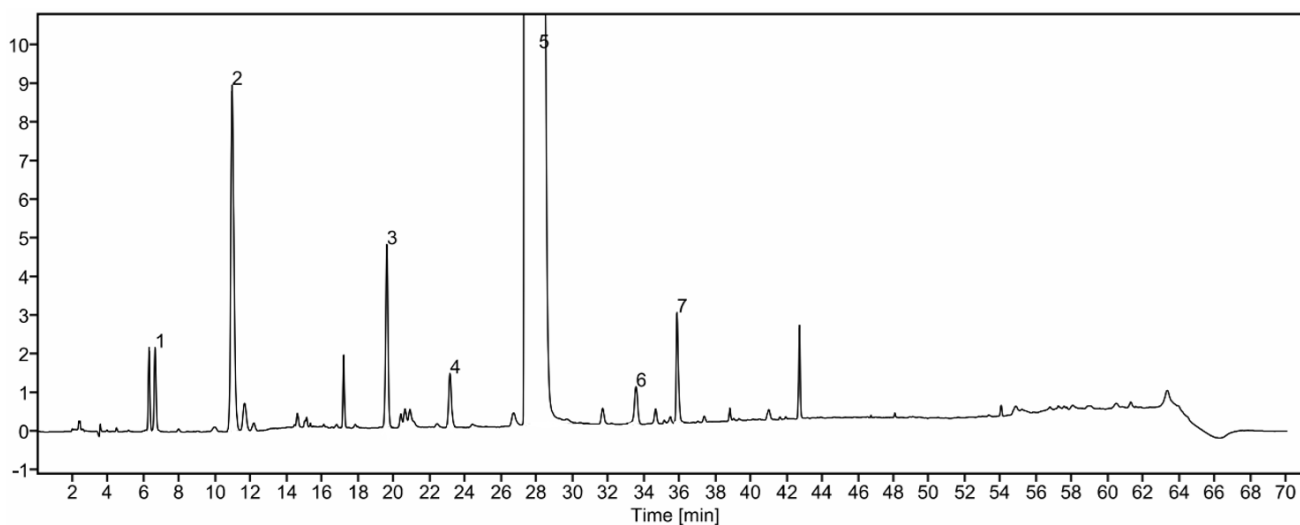


Peak ID: 1: Impurity 1; 2: Impurity 2; 3: Impurity P; 4: Impurity F; 5: Chlorhexidine

Column	Dr. Maisch, ReproSil-Pur Basic C18 (250 mm x 4.6 mm, 5 µm)
Method Ref.	Related Substances for the Chlorhexidine Dental Gel monograph from BP 2024
Buffer	10% v/v Trifluoroacetic acid in acetonitrile
Mobile Phase A	Buffer: acetonitrile: water (1.9:180:820, v/v)
Mobile Phase B	Buffer: water: acetonitrile (0.2:200:800, v/v/v)
Diluent	Mobile phase A
Flow rate	1.0 mL/min
Column Temp	40 °C
Injection Volume	25 µL
Detection	250 nm

Gradient	Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
	0 – 10	100	0	isocratic
	10 – 14	100 → 85	0 → 15	linear gradient
	14 – 43	85 → 75	15 → 25	linear gradient
	43 – 54	75 → 0	25 → 100	linear gradient
	54 – 60	0	100	isocratic
	60 – 62	0 → 100	100 → 0	linear gradient
	62 – 70	100	0	re-equilibration

Typical chromatogram for solution (5) in the Related Substances test for Chlorhexidine Mouthwash as published in BP 2024.

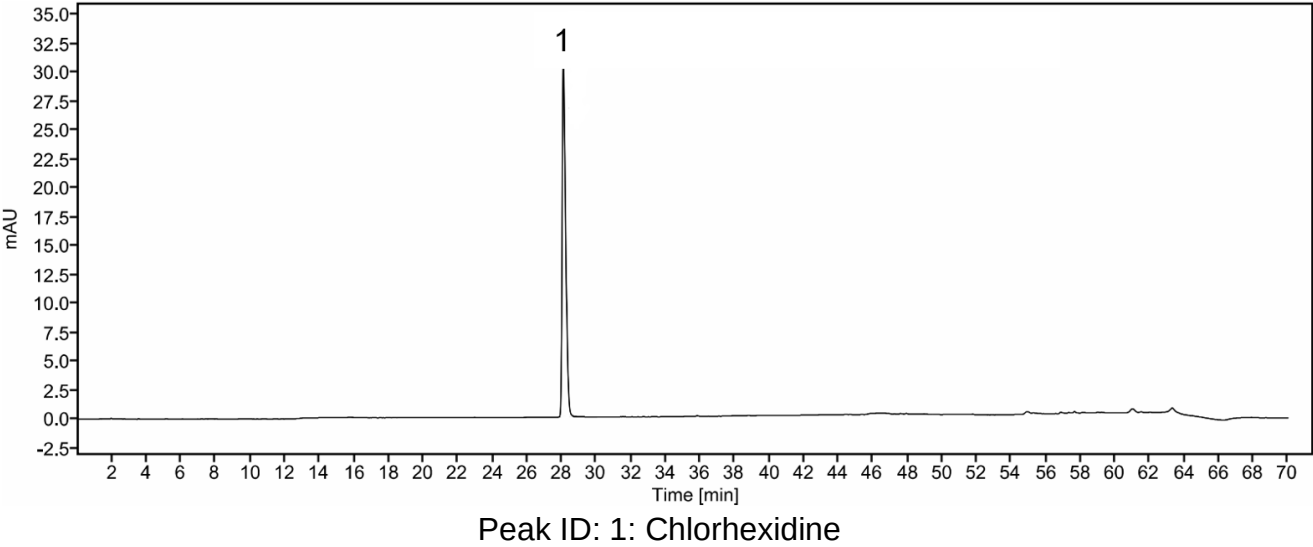


Peak ID: 1: Impurity G; 2: Impurity N; 3: Impurity F; 4: Impurity O; 5: Chlorhexidine; 6: Impurity J; 7: Impurity K

Column	Dr. Maisch, ReproSil-Pur Basic C18 (250 mm x 4.6 mm, 5 µm)
Method Ref.	Related Substances for the Chlorhexidine Mouthwash monograph from BP 2024
Buffer	10% v/v Trifluoroacetic acid in acetonitrile
Mobile	Buffer: acetonitrile: water (1.8:180:830, v/v/v)

Phase A				
Mobile Phase B	Buffer: water: acetonitrile (0.2:200:800, v/v/v)			
Diluent	Mobile phase A			
Flow rate	1.0 mL/min			
Column Temp	40 °C			
Injection Volume	25 µL			
Detection	250 nm			
Gradient	Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
	0 – 8	100	0	isocratic
	8 – 12	100 → 87	0 → 13	linear gradient
	12 – 20	87 → 86	13 → 14	linear gradient
	20 – 22	86	14	isocratic
	22 – 30	86 → 81	14 → 19	linear gradient
	30 – 40	81 → 60	19 → 40	linear gradient
	40 – 54	60 → 0	40 → 100	linear gradient
	54 – 60	0	100	isocratic
	60 – 62	0 → 100	100 → 0	linear gradient
	62 – 70	100	0	re-equilibration

Typical chromatogram for solution (2) in the Assay test for Chlorhexidine Mouthwash as published in BP 2024.



Column	Dr. Maisch, ReproSil-Pur Basic C18 (250 mm x 4.6 mm, 5 µm)
Method Ref.	Assay for the Chlorhexidine Mouthwash monograph from BP 2024
Buffer	10% v/v Trifluoroacetic acid in acetonitrile
Mobile Phase A	Buffer: acetonitrile: water (1.8:180:830, v/v/v)
Mobile Phase B	Buffer: water: acetonitrile (0.2:200:800, v/v/v)
Diluent	Mobile phase A
Flow rate	1.0 mL/min
Column Temp	40°C
Injection Volume	25 µL
Detection	250 nm

Gradient	Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
	0 – 8	100	0	isocratic
	8 – 12	100 → 87	0 → 13	linear gradient
	12 – 20	87 → 86	13 → 14	linear gradient
	20 – 22	86	14	isocratic
	22 – 30	86 → 81	14 → 19	linear gradient
	30 – 40	81 → 60	19 → 40	linear gradient
	40 – 54	60 → 0	40 → 100	linear gradient
	54 – 60	0	100	isocratic
	60 – 62	0 → 100	100 → 0	linear gradient
	62 – 70	100	0	re-equilibration