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Mesalazine Enema

General Notices

Mesalazine Rectal Suspension

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Enema is a *rectal suspension* containing Mesalazine in a suitable vehicle.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Filter a quantity of the enema containing 1.0 g of Mesalazine and discard the filtrate. Dry the residue at 110°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mesalazine *(RS 454)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix a quantity of the enema containing 1 g of Mesalazine with 700 mL of 0.01M *hydrochloric acid* for 10 minutes with the aid of ultrasound, add sufficient 0.01M *hydrochloric acid* to produce 1 L, mix and filter through a 0.45-µm membrane filter.
(2) Dilute 1 volume of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 10 volumes with 0.01M *hydrochloric acid*.
(3) 0.1% w/v of *mesalazine for system suitability EPCRS* in 0.01M *hydrochloric acid*.
(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O), *3-nitrosalicylic acid* (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).
(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 240 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide*.

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R, in the chromatogram obtained with solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurity C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phases.

(1) To a weighed quantity of the enema containing 1 g of Mesalazine, add 400 mL of mobile phase A and mix for 10 minutes with the aid of ultrasound with occasional shaking. Add sufficient mobile phase A to produce 1 L, mix and filter through a 0.45-µm membrane filter.
(2) 0.00002% w/v of *2-aminophenol* (impurity C) in mobile phase A.
(3) To 1 volume of solution (1) add sufficient of mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v of *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18e is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 220 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.22% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* *in* *water*.

Mobile phase B 0.17% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* in *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (200 ppm).

Impurity K

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 drops of 1M *sodium hydroxide* to a quantity of the enema containing 50 mg of Mesalazine, add 15 mL of the mobile phase, mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 25 mL and filter through a 0.45-µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP18e is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 205 nm.
(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of *methanol R2* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v of *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the enema containing 25 mg of Mesalazine, add 15 mL of 0.1M *hydrochloric acid* and mix for 50 minutes with the aid of ultrasound, with additional vortex mixing at 10 minute intervals. Add sufficient 0.1M *hydrochloric acid* to produce 25 mL, mix and filter. To 1 volume of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 50 volumes.
(2) 0.002% w/v of *mesalazine BPCRS* prepared by dissolving in 0.1M *hydrochloric acid* with the aid of ultrasound.
(3) Prepare a 0.01% w/v solution of *3-aminosalicylic acid* in 0.1M *hydrochloric acid* and dilute 1 volume of this solution to 100 volumes with a 0.1% w/v solution of *mesalazine BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature 40°.
(e) Use a detection wavelength of 240 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide solution*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the enema, Appendix V G, and calculate the content of $C_7H_7NO_3$ in the enema using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E, F, G, H, J, K, L, M, N, O, P, Q, R and S listed under Mesalazine.