

sirable, especially on large scale. Therefore, there is a need for improved methods, that avoid using strong acids, for separating the (1R,1'R)-atracurium besylate isomer mixture, particularly on a large scale. The present invention provides such methods.

BRIEF SUMMARY OF THE INVENTION

The present invention provides an improved chromatographic method of separating the isomers of (1R,1'R)-atracurium salts (e.g. the besylate salt), i.e., (1R-cis,1'R-cis), (1R-cis,1'R-trans), and (1R-trans,1'R-trans) isomers, which avoids the use of strong acids. Good separation as well as improved stability of the 1R-cis,1'R-cis cisatracurium besylate isomer can be achieved in accordance with the present invention. The method of chromatographically separating the (1R,1'R)-atracurium salts (e.g. the besylate salt) isomer mixture uses mixtures of an organic solvent, a polar aprotic co-solvent and a weak acid as eluent. In a preferred embodiment of the present invention, the weak acid is an organic acid. The improved chromatographic method of separating the (1R,1'R)-atracurium salts (e.g., the besylate salt) isomer mixture provides also the elimination of other substances.

The method of separating the isomers of (1R,1'R)-atracurium salts (e.g., the besylate salt) preferably includes the steps of:

- (a) dissolving an isomer mixture of (1R,1'R)-atracurium salt (e.g., the besylate salt) in at least one organic solvent;
- (b) applying the isomer mixture solution to an HPLC column containing a suitable stationary phase;
- (c) eluting the column with a mobile phase containing an organic solvent, a polar aprotic co-solvent and a weak acid;
- (d) collecting the fractions containing the 1R-cis,1'R-cis isomer;
- (e) optionally performing an ion exchange step to introduce the besylate anion; and
- (f) isolating the 1R-cis,1'R-cis isomer (e.g., cisatracurium besylate).

The present invention additionally provides a method for isolating 1R-cis,1'R-cis isomer of atracurium besylate obtained in accordance with the separation process of the present invention. The method for isolating the 1R-cis,1'R-cis isomer of atracurium besylate preferably includes the steps of:

- (a) combining eluted fractions, which preferably contain at least 90% of the 1R-cis,1'R-cis isomer;
- (b) washing the combined eluted fractions with acidic brine and separating the phases;
- (c) drying the organic phase and evaporating the solvent to obtain a residual oil;
- (d) dissolving the oil in a solvent; and
- (e) optionally isolating the product, e.g., by lyophilizing the solution or by precipitation.

The method for isolating the 1R-cis,1'R-cis isomer of cisatracurium besylate further optionally includes performing an ion exchange, e.g., via a strong anion exchange column or cartridge (hereinafter SAX).

A particularly preferred embodiment includes performing high-performance liquid chromatography (HPLC) separation using a column packed with silica, eluting with a mobile phase, which includes a mixture of an organic solvent in combination with a polar aprotic co-solvent and a weak acid, e.g., dichloromethane, DMSO and formic acid. Preferably, the dichloromethane:DMSO:formic acid ratio in the eluent mixture is about 70-85 (dichloromethane):8-20 (DMSO):5-

15 (formic acid). Exemplary eluent mixtures contain dichloromethane, DMSO and formic acid in ratios of e.g., 75:15:10, 78:13:9 and 80:10:10.

In accordance with the present invention, the desired 1R-cis,1'R-cis isomer can be obtained in high isomeric purity, e.g., greater than 99.5%.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the stability of the 1R-cis,1'R-cis isomer in the eluent mixture at 25° C. as a function of time.

FIG. 2 depicts the chromatogram of (1R,1'R)-atracurium besylate isomer mixture prepared in accordance with the present invention.

FIG. 3 depicts the chromatogram of (1R,1'R)-atracurium besylate isomer mixture using N-methyl-2-pyrrolidone as polar aprotic co-solvent in the eluent mixture.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an improved chromatographic method of separating the (1R,1'R)-atracurium salts (e.g., the besylate salt) isomer mixture, which avoids the use of strong acids in the eluent mixture. The method of the present invention allows for the separation of the 1R-cis,1'R-cis isomer from an isomer mixture of (1R,1'R)-atracurium salt (e.g., the besylate salt) and other compounds present.

The inventors of the present invention have found that the 1R-cis,1'R-cis isomer, obtained by HPLC separation as described in U.S. '510 and U.S. '978, is unstable in the eluent mixture and leads to the formation of decomposition products. Surprisingly, it has been further found by the inventors of the present invention, that good separation as well as improved stability of the 1R-cis,1'R-cis isomer can be achieved when the eluent contains a polar aprotic co-solvent and a weak acid, which is preferably an organic acid, thus the use of strong acid can be avoided.

The term "strong acids" refers to acids that dissociate practically completely (>99%) in aqueous solutions at standard temperature and pressure, such as benzenesulfonic acid, having a pKa of 0 and lower, while the term "weak acids" refers to acids that do not dissociate completely in aqueous solutions, such as acetic acid, having a pKa value of 2.5 or higher.

The term "(1R,1'R) atracurium besylate isomer mixture" refers to a mixture of the (1R-cis,1'R-cis), (1R-cis,1'R-trans) and (1R-trans,1'R-trans) isomers, while the term "cisatracurium besylate" refers to the 1R-cis,1'R-cis isomer.

The term "isomeric purity" as used herein, refers to the area percent of the peak corresponding to the 1R-cis,1'R-cis isomer relative to the total area percent of the (1R-cis,1'R-cis), (1R-cis,1'R-trans) and (1R-trans,1'R-trans) isomers.

In one embodiment, the present invention provides a method of separating isomers of (1R,1'R)-atracurium salt (e.g., the besylate salt), which includes the steps of:

- (a) dissolving the (1R,1'R)-atracurium salt (e.g., the besylate salt) isomer mixture in at least one organic solvent;
- (b) applying the (1R,1'R)-atracurium salt (e.g., the besylate salt) isomer mixture to an HPLC column containing a suitable stationary phase;
- (c) eluting the column with a mobile phase containing an organic solvent, a polar aprotic co-solvent and a weak acid;
- (d) collecting the fractions containing the 1R-cis,1'R-cis isomer;
- (e) optionally performing an ion exchange step as needed to introduce the besylate anion; and
- (f) isolating the 1R-cis,1'R-cis isomer (e.g., cisatracurium besylate).