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(54) **PURE DARIFENACIN HYDROBROMIDE SUBSTANTIALLY FREE OF OXIDIZED DARIFENACIN AND SALTS THEREOF AND PROCESSES FOR THE PREPARATION THEREOF**

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(57) **ABSTRACT**

Provided are darifenacin hydrobromide free of oxidized darifenacin, and processes for the preparation thereof.

PURE DARIFENACIN HYDROBROMIDE SUBSTANTIALLY FREE OF OXIDIZED DARIFENACIN AND SALTS THEREOF AND PROCESSES FOR THE PREPARATION THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

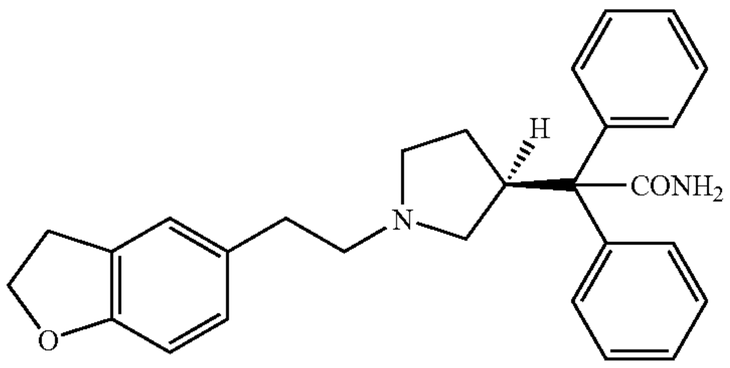
[0001] This application is a division of U.S. patent application Ser. No. 11/646,915, filed Dec. 27, 2006, which claims the benefit of priority to U.S. provisional application Ser. Nos. 60/754,395, filed Dec. 27, 2005; 60/772,250, filed Feb. 9, 2006; 60/776,311, filed Feb. 23, 2006; 60/809,147, filed May 25, 2006; 60/813,579, filed Jun. 8, 2006; 60/836,557, filed Aug. 8, 2006; 60/837,407, filed Aug. 10, 2006; 60/850,184, filed Oct. 5, 2006; 60/859,332, filed Nov. 15, 2006; and 60/873,680, filed Dec. 7, 2006, hereby incorporated by reference. This application is also related to U.S. application Ser. Nos. 11/647,109 and 11/646,919, each filed Dec. 27, 2006 and entitled "Processes for Preparing Darifenacin Hydrobromide," hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] The invention encompasses substantially pure darifenacin hydrobromide free of oxidized darifenacin and salts thereof, and processes for the preparation thereof.

BACKGROUND OF THE INVENTION

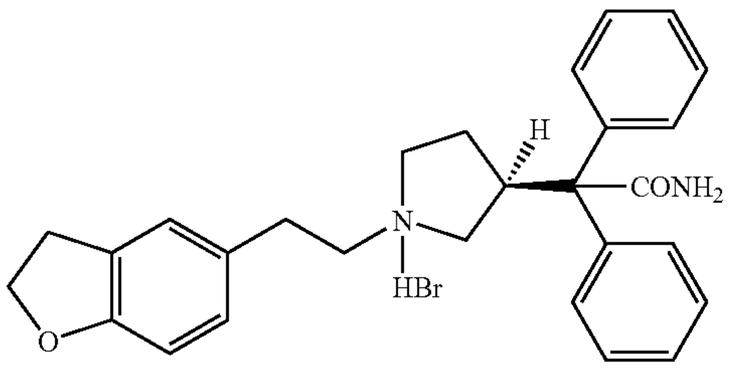
[0003] Darifenacin, (S)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide, a compound having the chemical structure,



Darifenacin

is a selective M3 receptor antagonist. Blockade of destructor muscle activity manifests in an increase in urine volume that the bladder can contain, reduction of urination frequency, and decrease in pressure and urgency associated with the urge to urinate, and thereby episodes of incontinence are reduced.

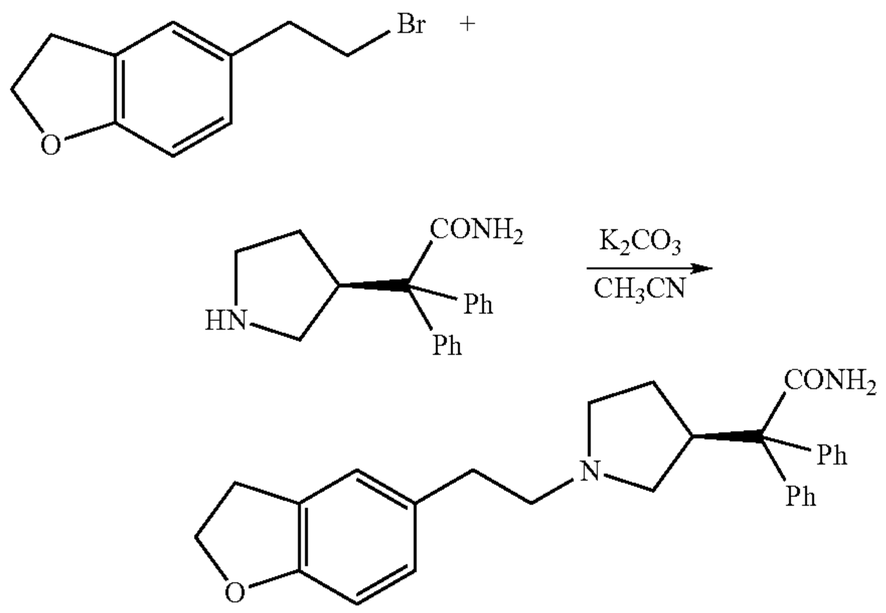
[0004] Darifenacin is administered as the hydrobromide salt, (S)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide hydrobromide, of the structure



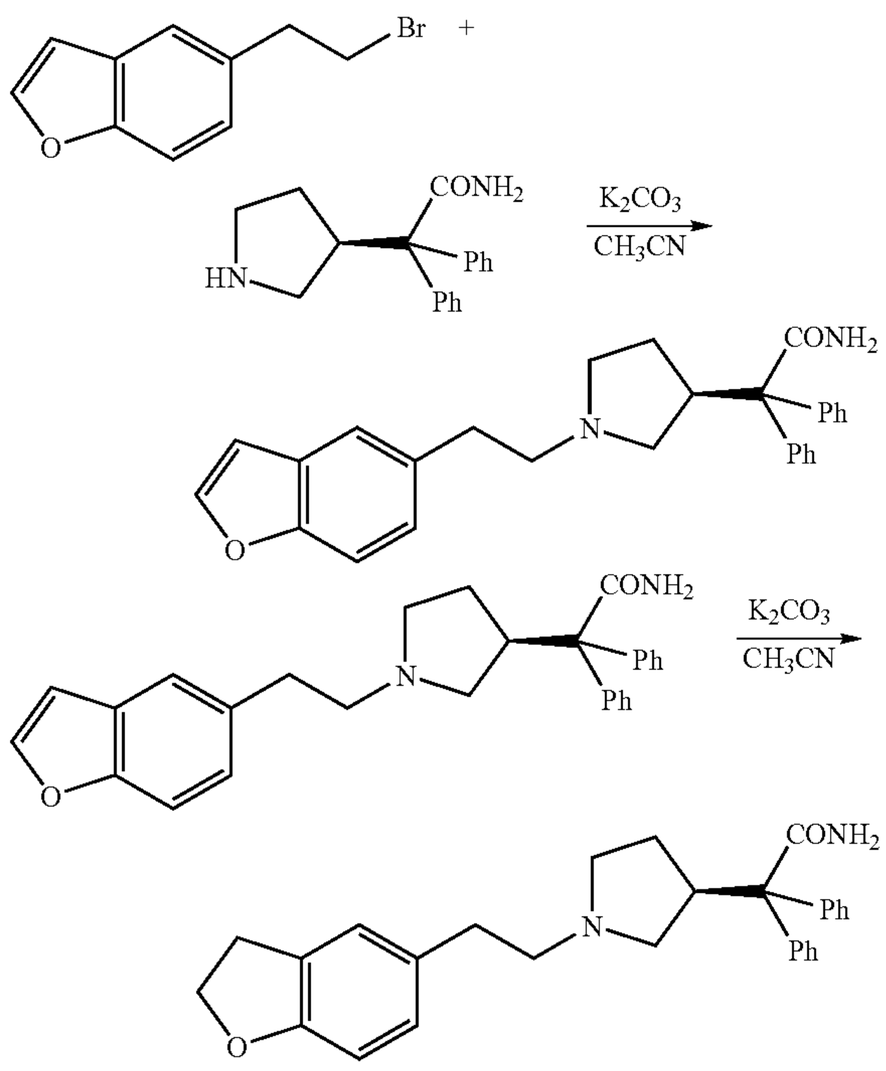
[0005] Darifenacin hydrobromide, and is marketed under the trade name ENABLEX® by Novartis.

[0006] Darifenacin hydrobromide and three routes for its preparation are disclosed in U.S. Pat. No. 5,096,890. The routes are illustrated by the following scheme:

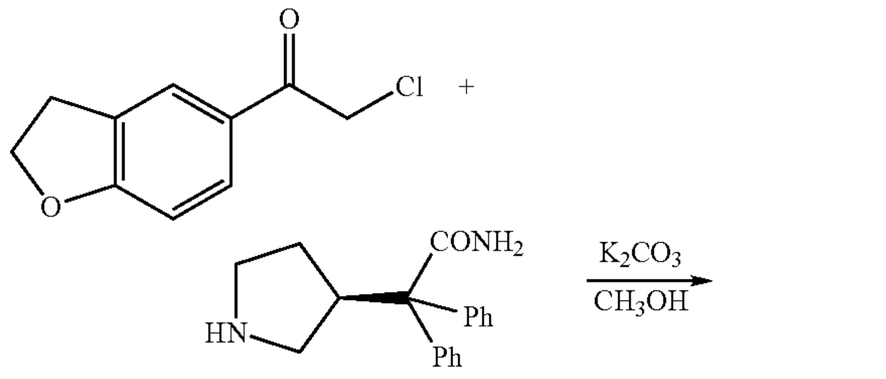
Route A:



Route B:



Route C:



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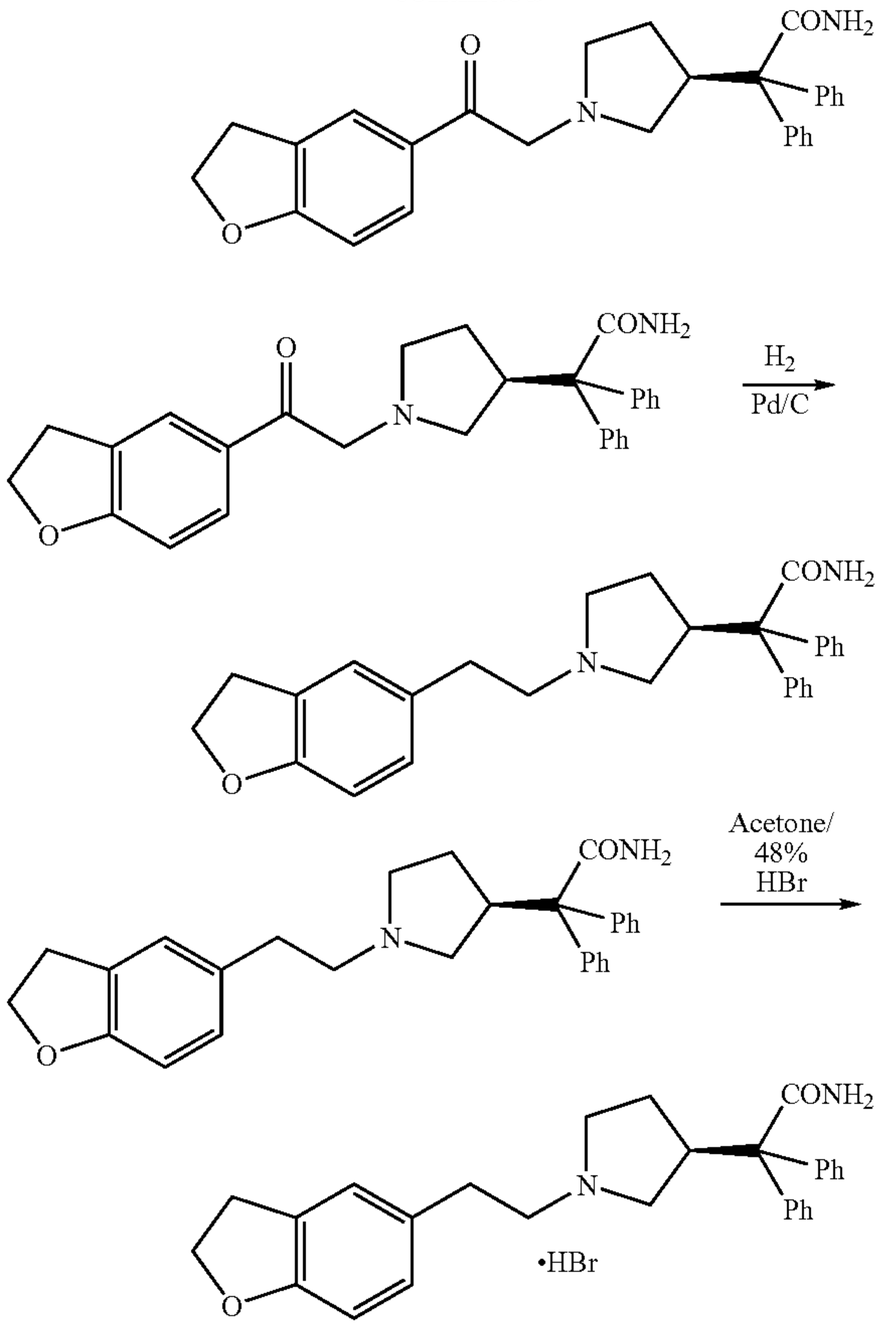
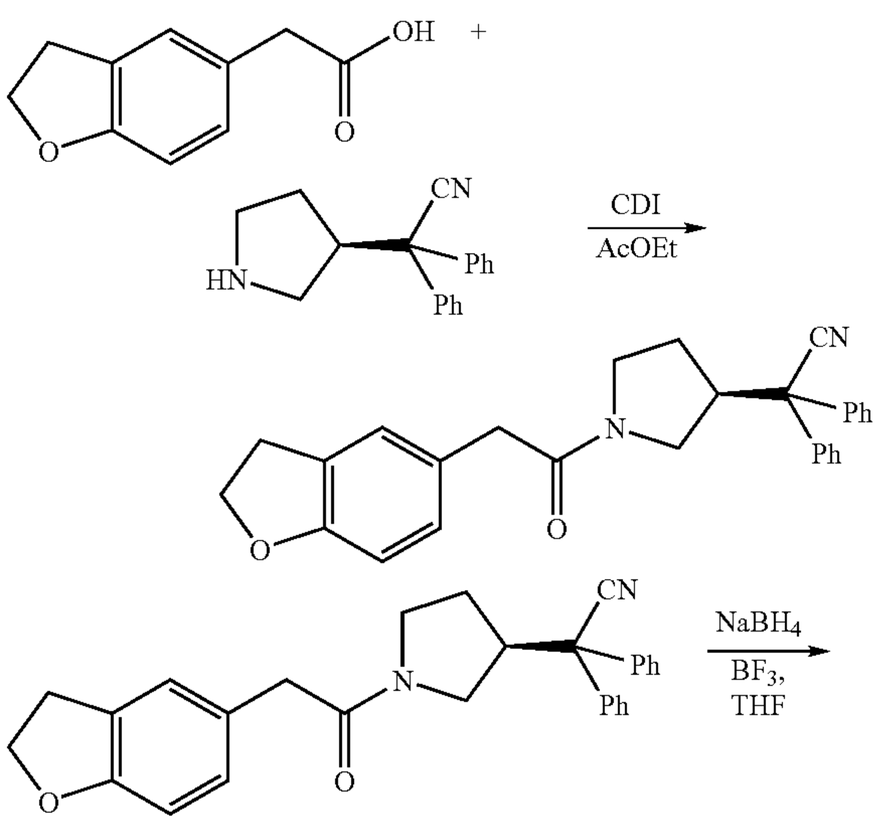


[0007] Darifenacin free base is purities by column chromatography on silica gel, and then converted to darifenacin hydrobromide by dissolving it in acetone and reacting with 48% hydrobromic acid.

[0008] Another process for preparing darifenacin HBr is disclosed in U.S. publication No. 2003/0191176, and is illustrated by the following scheme:



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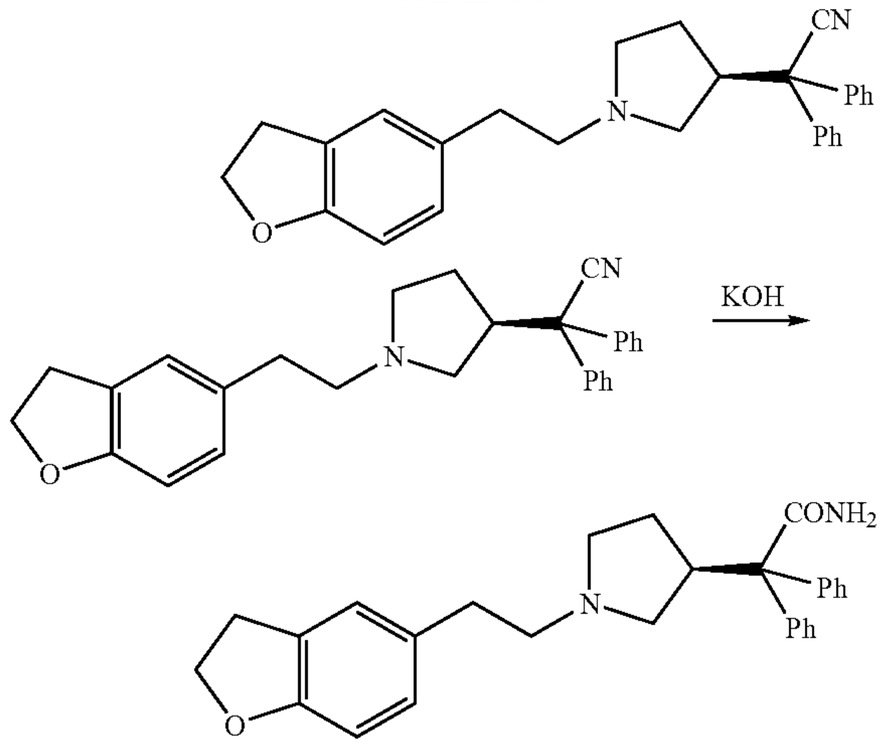


[0009] Darifenacin free base is purified by crystallization from acetonitrile-water mixture providing hydrate form of Darifenacin and from toluene providing toluene solvate of Darifenacin. Both purified darifenacin free base forms (hydrate or toluene solvate) are then converted to Darifenacin HBr by dissolving them in butan-2-one and adding 48% hydrobromic acid.

[0010] Like any synthetic compound, darifenacin hydrobromide can contain extraneous compounds or impurities. These impurities may be, for example, starting materials, by-products of the reaction, products of side reactions, or degradation products. Impurities in darifenacin hydrobromide, or any active pharmaceutical ingredient ("API"), are undesirable and, in extreme cases, might even be harmful to a patient being treated with a dosage form containing the API.

[0011] The purity of an API produced in a manufacturing process is critical for commercialization. The U.S. Food and Drug Administration ("FDA") requires that process impurities be maintained below set limits. For example, in its ICH Q7A guidance for API manufacturers, the FDA specifies the quality of raw materials that may be used, as well as acceptable process conditions, such as temperature, pressure, time, and stoichiometric ratios, including purification steps, such as crystallization, distillation, and liquid-liquid extraction. See ICH Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, Q7A, Current Step 4 Version (Nov. 10, 2000).

[0012] The product of a chemical reaction is rarely a single compound with sufficient purity to comply with pharmaceutical standards. Side products and by-products of the reaction and adjunct reagents used in the reaction will, in most cases, also be present in the product. At certain stages during processing of an API, such as darifenacin hydrobromide, it must be analyzed for purity, typically, by high performance liquid chromatography ("HPLC") or thin-layer chromatography ("TLC"), to determine if it is suitable for continued processing and, ultimately, for use in a pharmaceutical product. The FDA requires that an API is as free of impurities as possible, so that it is as safe as possible for clinical use. For example, the FDA recommends that the amounts of some impurities be limited to less than 0.1 percent. See ICH Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, Q7A, Current Step 4 Version (Nov. 10, 2000).

[0013] Generally, side products, by-products, and adjunct reagents (collectively "impurities") are identified spectroscopically and/or with another physical method, and then associated with a peak position, such as that in a chromatogram, or a spot on a TLC plate. See Strobel, H.A., et al., CHEMICAL INSTRUMENTATION: A SYSTEMATIC APPROACH, 953, 3d ed. (Wiley & Sons, New York 1989). Once a particular impurity has been associated with a peak position, the impurity can be identified in a sample by its relative position in the chromatogram, where the position in the chromatogram is measured in minutes between injection of the sample on the column and elution of the impurity through the detector. The relative position in the chromatogram is known as the "retention time."

[0014] The retention time can vary about a mean value based upon the condition of the instrumentation, as well as many other factors. To mitigate the effects such variations have upon accurate identification of an impurity, practitioners often use "relative retention time" ("RRT") to identify impurities. See supra Strobel at 922. The RRT of an impurity is calculated by dividing the retention time of the impurity by the retention time of a reference marker. The reference marker may be the API in which the impurity is present, or may be another compound that is either present in or added to the sample. A reference marker should be present in the sample in an amount that is sufficiently large to be detectable, but not in an amount large enough to saturate the column.

[0015] Those skilled in the art of drug manufacturing research and development understand that a relatively pure compound can be used as a "reference standard." A reference standard is similar to a reference marker, except that it may be used not only to identify the impurity, but also to quantify the amount of the impurity present in the sample.

[0016] A reference standard is an "external standard," when a solution of a known concentration of the reference standard and an unknown mixture are analyzed separately using the same technique. See supra Strobel at 924; Snyder, L. R., et al., INTRODUCTION TO MODERN LIQUID CHROMATOGRAPHY, 549, 2d ed. (John Wiley & Sons, New York 1979). The amount of the impurity in the sample can be determined by comparing the magnitude of the detector response for the reference standard to that for the impurity. See U.S. Pat. No. 6,333,198, hereby incorporated by reference.

[0017] The reference standard can also be used as an "internal standard," i.e., one that is directly added to the sample in a predetermined amount. When the reference standard is an internal standard, a "response factor," which compensates for differences in the sensitivity of the detector to the impurity and the reference standard, is used to quantify the amount of the impurity in the sample. See supra Strobel at 894. For this purpose, the reference standard is added directly to the mixture, and is known as an "internal standard." See supra Strobel at 925; Snyder at 552.

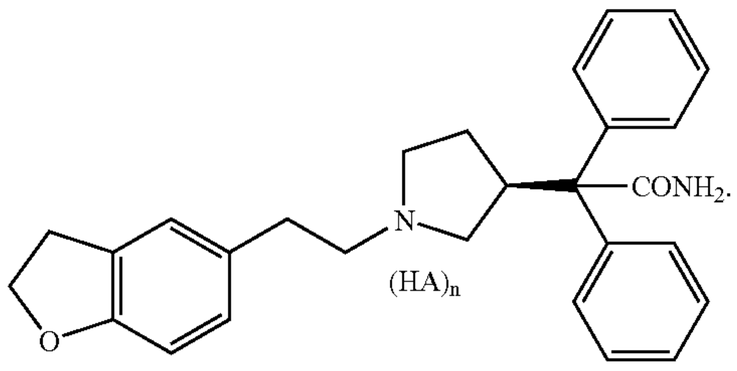
[0018] The technique of "standard addition" can also be used to quantify the amount of the impurity. This technique is used where the sample contains an unknown detectable amount of the reference standard. In a "standard addition," at least two samples are prepared by adding known and differing amounts of the internal standard. See supra Strobel at 391-393; Snyder at 571-572. The proportion of the detector response due to the reference standard present in the sample can be determined by plotting the detector response against the amount of the reference standard added to each of the samples, and extrapolating the plot to zero. See supra Strobel at 392, FIG. 11.4. The response of a detector in HPLC (e.g., UV detectors or refractive index detectors) can be and typically is different for each compound eluting from the HPLC column. Response factors, as known, account for this difference in the response signal of the detector to different compounds eluting from the column.

[0019] As is known by those skilled in the art, the management of process impurities is greatly enhanced by understanding their chemical structures and synthetic pathways, and by identifying the parameters that influence the amount of impurities in the final product.

[0020] Thus, providing substantially pure darifenacin hydrobromide, preferably, free of oxidized Darifenacin and salts thereof, and means for preparation thereof is beneficial.

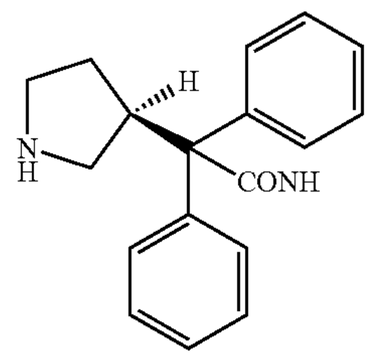
SUMMARY OF THE INVENTION

[0021] In one embodiment, the invention encompasses darifenacin hydrobromide having less than 0. 1% of oxidized Darifenacin and salts thereof of the following formula



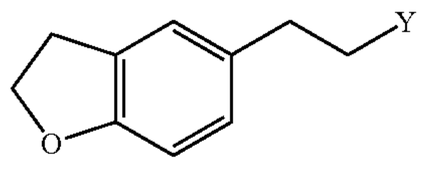
wherein n is either 0 or 1 and HA is an acid. Preferably, HA is HBr.

[0022] In another embodiment, the present invention encompasses a process for preparing darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof comprising a) combining 3-(S)-(+)-(1-carbamoyl-1, 1- diphenylmethyl)pyrrolidine tartrate or the free base derivative of the following formula,



XI

the compound of the following formula II,



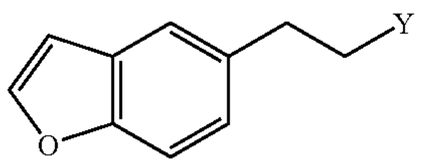
II

having less than 0.25% of compound of formula I, a solvent selected from the group consisting of a C_{6-9} aromatic hydrocarbon, a polar organic solvent, water, and mixtures thereof,

and a base to form a mixture; and b) admixing HBr with the mixture to obtain darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof; wherein Y is a leaving group selected from the group consisting of Cl , I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy.

[0023] In another embodiment, the invention encompasses a HPLC method for determining the presence and amount of oxidized Darifenacin and salts thereof in a sample of darifenacin hydrobromide comprising: a) combining a sample of darifenacin hydrobromide with a mixture of acetonitrile:water in a ratio of about 1: 1, to obtain a solution; b) injecting the solution into a column, preferably, a 150×4.6 mm×0.5 μm Phenyl C6 column; c) eluting the sample from the column using a mixture of acetonitrile:water in a ratio of 9:1 (referred to as eluent A) and buffer (referred to as eluent B) as an eluent; and d) measuring the oxidized darifenacin and salts thereof content in the sample with a UV detector.

[0024] In another embodiment, the invention encompasses a compound of the following formula I



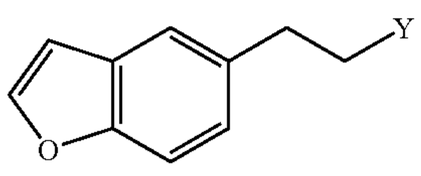
I

wherein Y is a leaving group selected from the group consisting of Cl , I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy.

[0025] In one embodiment, the present invention encompasses a process of determining the presence of the compound of formula I in a sample comprising the compound of formula I and the compound of formula II by a process comprising carrying out HPLC or TLC with the compound of formula I as a reference marker.

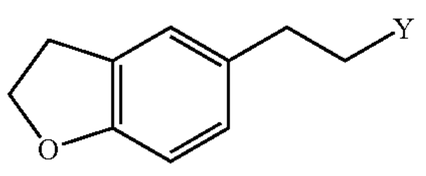
[0026] In another embodiment, the present invention encompasses a process of determining the amount of the compound of formula I in a sample comprising the compound of formula I and the compound of formula II by a process comprising carrying out HPLC with the of formula I as a reference standard.

[0027] In yet another embodiment, the invention encompasses an HPLC method for determining the presence and the amount of a compound of formula I



I

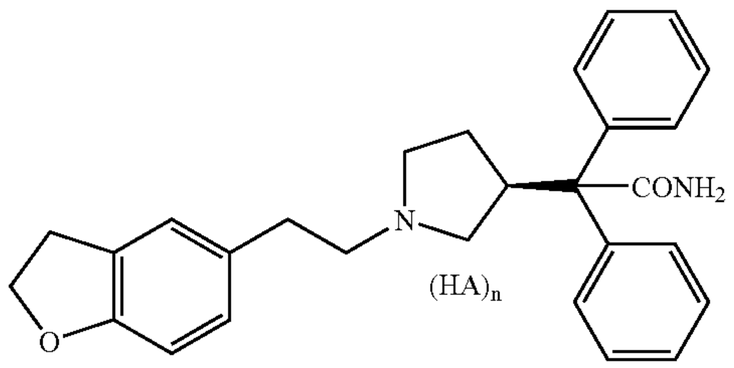
in a sample of a compound of formula II



II

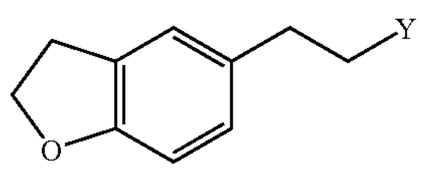
comprising: (a) combining a sample of a derivative of ethyl-dihydrobenzofuran of formula I with a mixture of acetonitrile:water in a ratio of about 1:1, to obtain a solution; (b) injecting the solution into a column, preferably, a 250×4.6 mm 0.5 μrm C18 column; (c) eluting the sample from the column using a mixture of acetonitrile and buffer as an eluent; and (d) measuring the amount of the compound of formula I in the sample with a UV detector, wherein Y is a leaving group selected from the group consisting of Cl , I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy.

[0028] In one embodiment, the invention encompasses a process for preparing darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof of the following formula



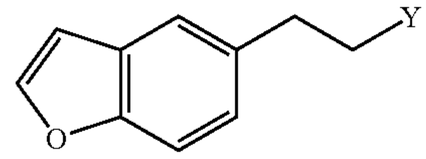
comprising: a) obtaining one or more samples of one of one or more batches of a compound of formula II;

II



b) measuring the level of the compound of formula I

I



in one or more of the samples of one or more of the batches of step a); c) selecting a batch that has less than about 0.25% of the compound of formula I based upon the measurements of step b); and d) using the batch selected in step (c) to prepare the darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof, wherein Y is a leaving group selected from the group consisting of Cl , I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy, preferably, Cl , n is either 0 or 1, and HA is an acid. Preferably, HA is HBr.

[0029] In another embodiment, the invention encompasses a pharmaceutical composition comprising darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof and at least one pharmaceutically acceptable excipient.

[0030] In another embodiment, the invention encompasses a process for preparing the pharmaceutical composition, comprising combining darifenacin hydrobromide having less

than 0.1% of oxidized darifenacin and salts thereof and the pharmaceutically acceptable excipient.

[0031] In another embodiment, the invention encompasses a method of treating urinary incontinence reducing urgency and increasing urine volume that the bladder can contain comprising administering a a therapeutically effective amount of a pharmacuetical composition comprising darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof and at least one pharmaceutically acceptable excipient to a patient in need thereof.

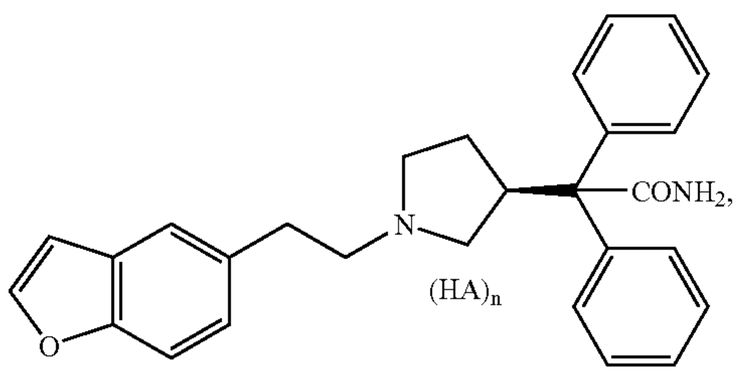
[0032] In another embodiment, the invention encompasses the use of darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof in the manufacture of a pharmaceutical composition for the treatment of urinary incontinence reducing urgency and increasing urine volume that the bladder can contain.

DETAILED DESCRIPTION OF THE INVENTION

[0033] The present invention is directed to substantially pure Darifenacin-HBr. Especially, the invention is directed to Darifenacin-HBr free of oxidized Darifenacin and salts thereof, as well as processes for its preparation.

[0034] Oxidized Darifenacin and salts thereof are impurities that have been difficult to separate from darifenacin hydrobromide by conventional methods because of their close structural similarity to darifenacin hydrobromide.

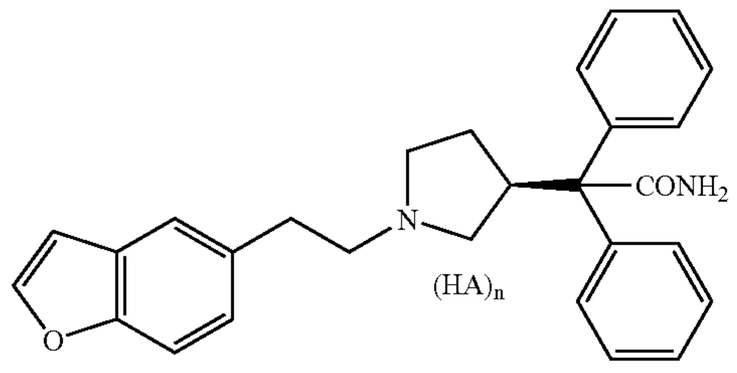
[0035] As used herein, unless otherwise defined, the term "free of oxidized Darifenacin and salts thereof" when referring to darifenacin hydrobromide means darifenacin hydrobromide having very low levels of oxidized Darifenacin and salts thereof of the following formula,



and preferably less than 0.1% of oxidized darifenacin and salts thereof; wherein n is either 0 or 1 and HA is an acid. Preferably, HA is HBr.

[0036] The level of oxidized Darifenacin and salts thereof in Darifenacin hydrobromide can be measured by w/w units. The measurement can be done by any method known to a skilled artisan, such as an HPLC method.

[0037] The invention encompasses darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof of the following formula



wherein n is either 0 or 1, wherein HA is an acid, preferably, HBr. Preferably, darifenacin hydrobromide has less than 0.08%, more preferably, less than 0.05% of oxidized darifenacin and salts thereof.

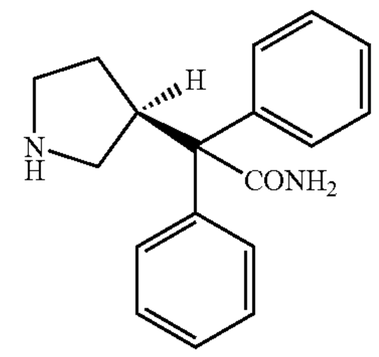
[0038] When n is 0, the above formula refers to oxidized Darifenacin.

[0039] When n is 1, the above formula refers to oxidized Darifenacin salt.

[0040] When n is 1 and HA is HBr, the above formula refers to oxidized Darifenacin HBr.

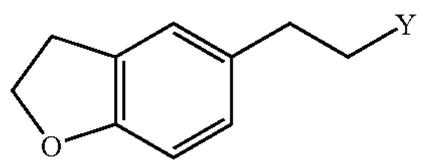
[0041] Darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof is prepared by a process comprising a) combining 3-(S)-(+)-(1-carbamoyl- 1,1-diphenylmethyl)pyrrolidine tartrate or the free base derivative of the following formula,

XI



the compound of the following formula II,

II



having less than 0.25% of the compound of formula I, a solvent selected from the group consisting of a C_{6-9} aromatic hydrocarbon, a polar organic solvent, water, and mixtures thereof, and a base to form a mixture; and b) admixing HBr with the mixture to obtain darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof; wherein Y is a leaving group selected from the group consisting of Cl , I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy, preferably, Cl.

[0042] Preferably, when the compound of formula II has less than 0.15% of the compound of formula I, the obtained Darifenacin hydrobromide has less than 0.08% of oxidized Darifenacin and salts thereof.

[0043] More preferably, when the compound of formula II has less than 0.1% of the compound of formula I, the obtained Darifenacin hydrobromide has less than 0.05% of oxidized Darifenacin and salts thereof.

[0044] Preferably, the polar aprotic organic solvent is selected from the group consisting of an amide, a C_{1-10} halogenated aliphatic hydrocarbon, a sulfoxide, an ester, a nitrile, and a ketone. A preferred amide is dimethylformamide (DMF). A preferred C_{1-0} halogenated aliphatic hydrocarbon is a C_{1-5} halogenated aliphatic hydrocarbon, more preferably, dichloromethane (DCM). Preferably, the sulfoxide is a C_{2-5} sulfoxide, more preferably, dimethylsulfoxide (DMSO). Preferably, the ester is a C_{2-5} ester, more preferably, ethyl acetate (EtOAc). A preferred ketone is a C_{3-6} ketone, more preferably, methyl ethyl ketone (MEK). Preferably, the nitrile

is a C_{24} nitrile, more preferably, acetontirile (ACN). Preferably, the C_{6-9} aromatic is toluene or xylene. Preferred mixtures are either that of toluene and water or that of DCM and water. The more preferred solvent is water.

[0045] The base may be an inorganic base or an organic base. A preferred organic base is selected from the group consisting of aliphatic and aromatic amines. Preferably, the aliphatic amine is triethylamine, tribytulamine, methylmorpholine, or N,N-diisopropylethyl amine. Preferably, the aromatic amine is pyridine. A preferred inorganic base is either alkali carbonate or alkali bicarbonate. Preferably, the alkali carbonate is sodium carbonate or potassium carbonate. Preferably, the alkali bicarbonate is either sodium bicarbonate or potassium bicarbonate. The most preferred base is an alkali carbonate, even most preferably, potassium carbonate.

[0046] Preferably, the mixture is heated to a temperature of about 50° C. to about reflux, and more preferably to a temperature of about 60° C. to about reflux, prior to admixing with HBr. Preferably, the mixture is maintained, under heating, for about 1 to about 5 hours, and more preferably for about 2 to about 3 hours. After maintaining the mixture, the mixture is cooled to a temperature of about 35° C. to about 15° C., and preferably to about 25° C. to about 15° C. Preferably, after cooling, an organic solvent selected from the group consisting of DCM, EtOAc, and butyl acetate is added to the mixture, to give a mixture having an aqueous phase and an organic phase. The phases are then separated, and HBr is admixed with the organic phase. Preferably, HBr is added to the organic phase.

[0047] Preferably, a small amount of an anhydride may be added to the organic phase, after separating the phases, followed by maintaining for about I to about 3 hours. After maintaining, the organic solvent is removed, and a C_{2-5} alcohol and hydrobromic acid are added, to obtain darifenacin hydrobromide. Preferably, the C_{2-5} alcohol is n-butanol, sec-butanol, ethanol, 2-methyl-2-butanol, or isopropanol, more preferably, n-butanol.

[0048] Darifenacin hydrobromide having less than 0.1%, preferably, less than 0.08%, more preferably, less than 0.05% of oxidized Darifenacin and salts thereof may be recovered by removing the residual water and the organic solvent from the acidic mixture obtained after the addition of HBr, preferably, by distillation under vacuum, to induce precipitation of the darifenacin hydrobromide. The mixture is then cooled to room temperature and the resulting precipitate of darifenacin hydrobromide is separated from the mixture by filtration.

[0049] Darifenacin hydrobromide may be further purified by crystallizing the recovered precipitate from a C_{2-5} alcohol. The process comprises suspending the precipitate in a C_{2-5} alcohol, heating the suspension to a temperature sufficient to induce dissolution of the darifenacin hydrobromide, and cooling the resulting solution to induce crystallization of the darifenacin hydrobromide. Prior to cooling, the solution may be purified with active charcoal. The crystallized product may be isolated by filtration, washing and drying. Preferably, the C_{2-5} alcohol is n-butanol, sec-butanol, ethanol, 2-methyl-2-butanol, or isopropanol, and more preferably n-butanol.

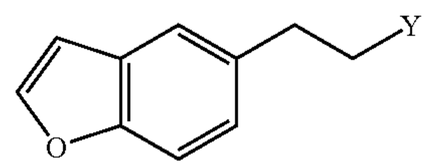
[0050] The presence and level of oxidized darifenacin and salts thereof in a darifenacin-HBr sample is determined by an HPLC method comprising: (a) combining a darifenacin-HBr sample with a mixture of acetonitrile:water in a ratio of about 1:1, to obtain a solution; (b) injecting the solution into a column, preferably, a 150×4.6 mm×0.5 μm Phenyl C6 column; (c) eluting the sample from the column using a mixture of acetonitrile:water in a ratio of 9:1 (referred to as eluent A) and buffer (referred to as eluent B) as an eluent; and (d) measuring the oxidized darifenacin content in the sample with a WV detector.

[0051] Preferably, the buffer used in this method is a phosphate buffer. The phosphate buffer comprises an aqueous solution of K_2HPO_4 having a pH of about 9.

[0052] Typically, the sample is eluted through the column by gradient elution. Preferably, the eluent is a mixture of eluent A and eluent B. More preferably, the sample is eluted through the column by gradient elution under the following conditions: At the time 0 minutes, the eluent contains 40% of eluent A and 60% of eluent B, at 20 minutes, the eluent contains 70% of eluent A and 30% of eluent B, and at 30 minutes, the eluent contains 70% of eluent A and 30% of eluent B.

[0053] Preferably, the presence and content of the oxidized darifenacin and salts thereof are measured at a wavelength of 215 nm.

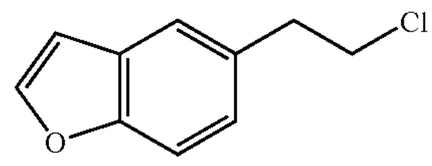
[0054] The invention also encompasses a compound of the of formula I;



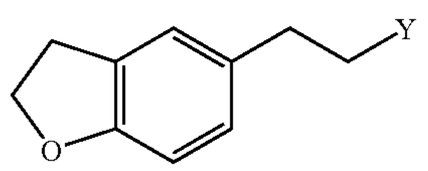
I

wherein Y is a leaving group selected from the group consisting of Cl, I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy. Preferably, Y is Cl.

[0055] When Y is Cl, the compound of formula I refers to 5-(2-chloroethyl)-2,3-benzofuran of the following formula.

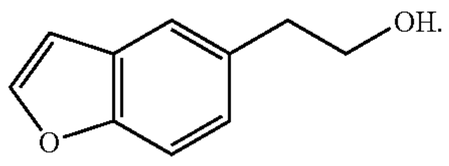


[0056] The compound of formula I can be prepared by any method known to one skilled in the art. Such methods include, but are not limited to, the method disclosed in U.S. Pat. No. 5,096,890, hereby incorporated by reference. Such methods also include reacting a the compound of formula II



II

with an oxidizing agent, as exemplified in example 5, or via 2-(benzofuran-5-yl)ethanol of formula V.



V

The 2-(benzofuran-5-yl)ethanol of formula V can be produced from commercially available 2-(benzofuran-5-yl)acetic acid, according to any method known to one skilled in the art. Such methods include, but are not limited to, converting the acid to the corresponding ester and reducing the ester to obtain the alcohol, as exemplified in examples 1-3.

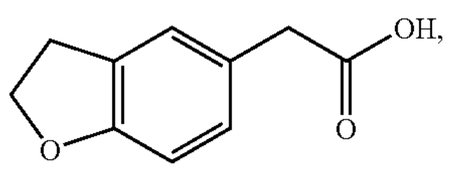
[0057] It is believed that oxidized Darifenacin and salts thereof are formed during the above-described synthesis of darifenacin-HBr by reaction of an the compound of formula I, which is an impurity often present in the starting compound of formula II, with the starting (S)-(1-carbamoyl-1,1-diphenylmethyl)pyrrolidine of formula XI or salt thereof.

[0058] Accordingly, because separating the oxidized Darifenacin and salts thereof from the darifenacin-HBr is difficult, a method for obtaining darifenacin-HBr free of oxidized Darifenacin and salts thereof would be of a great advantage.

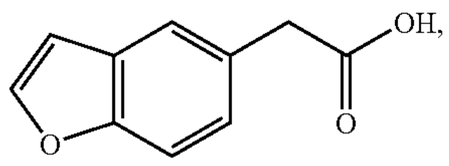
[0059] It was found that starting with compound of formula II having very low levels of the compound of formula I, and preferably less than 0.25% of the compound of formula I leads to darifenacin-HBr free of oxidized Darifenacin and salts thereof.

[0060] The level of the compound of formula I in the compound of formula II can be measured by area % units. The said measurement can be obtained by any method known to a skilled artisan, such as an HPLC method.

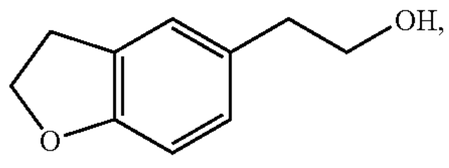
[0061] The compound of formula II having less than 0.25% of the compound of formula I can be prepared by the process disclosed in U.S. application Ser. No. 11/646,919, filed December 27, 2006 and entitled "Processes for Prepared Darifenacin Hydrobromide," wherein the starting commercially available acid analogue 2,3-dihydrobenzofuran-5-acetic acid



has less than 0.4% area by HPLC of 5-benzofuranacetic acid, providing



5-(2-hydroxyethyl)-2,3-dihydrobenzofuran of formula III



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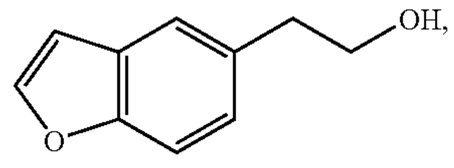
having less than 0.5% of 2-(benzoftiran-5-yl)ethanol of formula V,

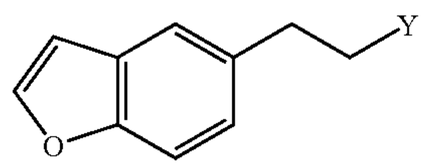


V

and the solvent is an aromatic hydrocarbon, more preferably, C_{6-9} aromatic hydrocarbon, most preferably toluene, as exemplified in examples 8 and 9.

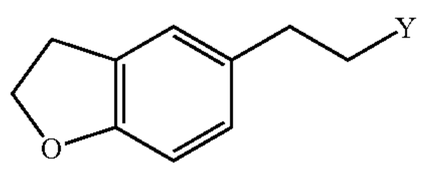
[0062] The present invention encompasses a process of determining the presence of the compound of formula I in a sample comprising the compound of formula I and the compound of formula II by a process comprising carrying out HPLC or TLC with the compound of formula I as a reference marker.

[0063] The above process comprises determining the relative retention time of a compound of formula I



I

in a sample of a compound of formula II



II

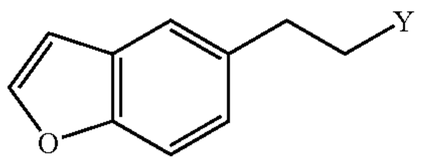
by a process comprising: (a) measuring by HPLC or TLC the relative retention time (referred to as RRT, or RRF, respectively) corresponding to a compound of formula I in a reference marker sample; (b) determining by HPLC or TLC the relative retention time corresponding to a compound of formula I in a sample comprising a compound of formula I and a compound of formula II; and (c) determining the relative retention time of the compound of formula I in the sample by comparing the relative retention time (RRT or RRF) of step (a) to the RRT or RRF of step (b), wherein Y is a leaving group selected from the group consisting of Cl, I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy, preferably, Cl.

[0064] The invention also encompasses a process of determining the amount of the compound of formula I in a sample comprising the compound of formula I and the compound of formula II by a process comprising carrying out an HPLC with the compound of formula I as a reference standard.

[0065] The above process comprises: (a) measuring by HPLC the area under a peak corresponding to a compound of formula I in a reference standard comprising a known amount of the compound of formula I; (b) measuring by HPLC the area under a peak corresponding to a compound of formula I in a sample comprising a compound of formula I and a compound of formula II; and (c) determining the amount of the compound of formula I in the sample by comparing the area of step (a) to the area of step (b), wherein Y is a leaving group

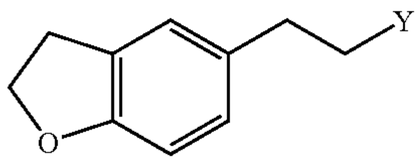
selected from the group consisting of Cl, I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyoxyl, and trifluoromethansulfonyloxy, preferably, Cl.

[0066] The HPLC method used to determine the presence and the presence and the amount of a compound of formula I



I

in a sample of a compound of formula II



II

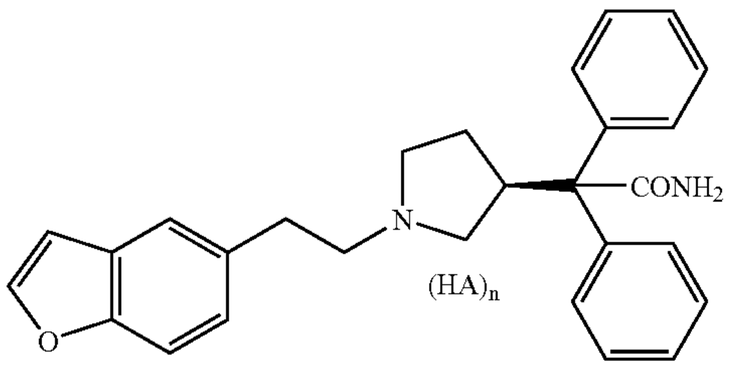
comprises: (a) combining a sample of a derivative of ethyldihydrobenzofuran of formula I with a mixture of acetonitrile:water in a ratio of about 1:1, to obtain a solution; (b) injecting the solution into a column, preferably, a 250×4.6 mm×0.5 μm C18 column; (c) eluting the sample from the column using a mixture of acetonitrile and buffer as an eluent; and (d) measuring the amount of the compound of formula I in the sample with a UV detector, wherein Y is a leaving group selected from the group consisting of Cl, I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy.

[0067] Preferably, the buffer used in this method is a phosphate buffer. The phosphate buffer comprises an aqueous solution of K_2HPO_4 having a pH of about 9.

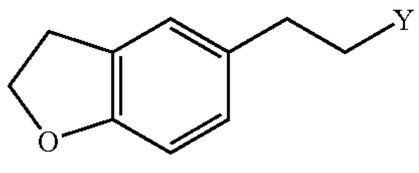
[0068] Typically, the sample is eluted through the column by gradient elution. Preferably, the eluent is a mixture of eluent A and buffer. More preferably, the sample is eluted through the column by gradient elution under the following conditions: At the time 0 minutes, the eluent contains 50% of eluent A and 50% of buffer, at 20 minutes, the eluent contains 70% of eluent A and 30% of buffer, and at 30 minutes, the eluent contains 70% of eluent A and 30% of buffer.

[0069] Preferably, the presence and content of the compound of formula I is measured at a wavelength of 215 nm.

[0070] The invention also encompasses a process for preparing darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof of the following formula

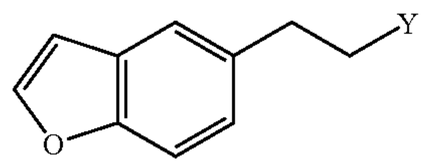


comprising: a) obtaining one or more samples of one of one or more batches of a compound of formula II;



II

b) measuring the level of a compound of formula I



I

in one or more of the samples of one or more of the batches of step a);

c) selecting a batch that has less than about 0.25% of the compound of formula I based upon the measurements of step b); and

d) using the batch selected in step (c) to prepare the darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof, wherein Y is a leaving group selected from the group consisting of Cl, I, brosyloxy, mesyloxy, tosyloxy, trifluoroacetyloxy, and trifluoromethansulfonyloxy; wherein n is either 0 or 1, wherein HA is an acid, preferably, HBr.

[0071] Typically, the one or more samples of compound of formula II of step (a) has less .than about 0.25% of the compound of formula I.

[0072] When the sample of the compound of formula II of step (a) has more than about 0.25% of the compound of formula I, according to the measurement in step (b), the sample may be purified, prior to performing step (c). The purification may be performed by at least one crystallization process or by column chromatogrpahy.

[0073] Typically, the purified sample compound of formula II has a lower level of compound of formula I than the level present before purification. Preferably, the compound of formula II sample of step (a) obtained after purification has less than about 0.25% of the compound of formula I.

[0074] The preparation of Darifenacin hydrobromide having less than 0.1% of oxidized Darifenacin and salts thereof from the selected batch of compound of formula II, can be done for example, by the process described before.

[0075] Unless specified otherwise, the darifenacin-HBr of step (d) of the above process may be in any physical form, including, for example, crystalline forms and amorphous forms.

[0076] The darifenacin-HBr having less than 0.10% of oxidized Darifenacin and salts thereof may be formulated into pharmaceutical compositions for the treatment of urinary incontinence reducing urgency and increasing urine volume that the bladder can contain.

[0077] The invention encompasses a pharmaceutical composition comprising darifenacin hydrobromide having less than 0.10% of oxidized Darifenacin and salts thereof, and at least one pharmaceutically acceptable excipient. Suitable excipients include, but are not limited to, diluents, carriers, fillers, bulking agents, binders, disintegrants, disintegration inhibitors, absorption accelerators, wetting agents, lubricants, glidants, surface active agents, flavoring agents, and the like. Selection of excipients and the amounts to use can be readily determined by an experienced formulation scientist in view of standard procedures and reference works known in the art.

[0078] The pharmaceutical composition can be formulated into a solid or a liquid dosage form for administration to a

patient. Dosage forms include, but are not limited to, tablets, capsules, powders, syrups, suspensions, emulsions, injection preparations, and the like.

[0079] The invention also encompasses a process for preparing a pharmaceutical composition comprising combining darifenacin hydrobromide having less than 0.10% of oxidized Darifenacin and salts thereof with at least one pharmaceutically acceptable excipient.

[0080] The invention also encompasses a method of treating urinary incontinence reducing urgency and increasing urine volume that the bladder can contain comprising administering a therapeutically effective amount of a pharmaceutical composition of darifenacin hydrobromide having less than 0.10% of oxidized Darifenacin and salts thereof, and at least one pharmaceutically acceptable excipient to a patient in need thereof.

[0081] The invention also encompasses use of darifenacin hydrobromide having less than 0.10% of oxidized Darifenacin and salts thereof in the manufacture of a pharmaceutical composition for the treatment of urinary incontinence reducing urgency and increasing urine volume that the bladder can contain.

[0082] Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one of ordinary skill in the art from consideration of the specification. The invention is further defined by reference to the following examples. It will be apparent to those of ordinary skill in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

EXAMPLES

Analytical Methods

[0083] (a) Impurities determination in darifenacin-HBr by HPLC analysis:

Column & Packing:	Phenyl-C6 150 mm × 4.6 mm × 5.0 μm
Buffer:	K_2HPO_4 0.02M pH 9.0: 3.48 g of K_2HPO_4 in 1000 mL of water; Filter on a 0.45 μm filter.
Eluent A:	acetonitrile/water- 90/10
Eluent B:	Buffer

Gradient	Time (min)	% Eluent B	% Eluent A
	0	60	40
	20	30	70
	30	30	70

Equilibrium time:	15 minutes
Sample volume:	5.0 μL
Flow Rate:	1.2 mL/min
Detector:	UV at 215 nm
Column temperature:	35° C.
Diluent	H_2O:Acetonitrile (50:50)

Typical retention times are:

Compound	Retention Time (minutes)	Relative Retention Time
(S)-(1-carbamoyl-1,1-diphenylmethyl)pyrrolidine (DIPAMP) of formula IV	2.6	0.19
5-(2-chloroethyl)-2,3-dihydrobenzofuran (DBF-EtCl) of formula II	12.6	0.91
Darifenacin	13.8	1.00
Oxidized darifenacin	16.0	1.16

The detection limit is 0.01%.

(b) Impurities determination in 5-(2-chloroethyl)-2,3-dihydrobenzofuran of Formula II by HPLC analysis:

Column & Packing:	C18 250 mm × 4.6 mm × 5.0 μm
Buffer:	K_2HPO_4 0.02M pH 7.0: 3.48 g of K_2HPO_4 in 1000 mL of deionized water, adjust pH at 7.0 ± 0.2 with H_3PO_4 15%(w/v). Filter on a 0.45 μm filter.
Eluent A:	Acetonitrile
Eluent B:	Buffer

Gradient	Time (min)	% Eluent B	% Eluent A
	0	50	50
	20	30	70
	30	30	70

Equilibrium time:	8 minutes
Sample volume:	5.0 μL
Flow Rate:	1.0 mL/min
Detector:	UV at 215 nm
Column temperature:	35° C.
Diluent	H_2O:Acetonitrile (50:50)

A typical Retention time of the 5-(2-chloroethyl)-2,3-dihydrobenzofiran of formula II is 12.7 min and a typical retention time of the oxidized impurity 1-(benzofuran-5-yl)ethyl chloride of formula I is 14.9 min. The detection limit is 0.01%.

(c) TLC analysis

[0084] TLC is performed with silica gel as the stationary phase and a mixture of hexane and toluene (95:5 vol:vol) as the eluent.

Example 1

[0085] Preparation of 2,3-dihydrobenzofuran-5-acetic acid methyl ester (DBFAcOMe)

[0086] 98% H_2SO_4 (2 g, 0.02 mol)) was added to a solution of 2,3-dihydrobenzofuran-5-acetic acid (DBFAcOH) (200g; 1.12 mol) in methanol (500 ml) and the mixture was refluxed for 3hrs. After cooling to room temperature, $NaHCO_3$ (6.7 g, 0.11 mol) was added to the reaction mixture and the solvent was distilled off at atmospheric pressure (about 440 ml) to give a light pink oily residue.

[0087] The oily residue was dissolved in toluene (250 ml) and washed with $NaHCO_3$ 6% (50 ml). After phase separation the solvent was eliminated under vacuum distillation obtaining an oily residue (227 g).

Example 2

[0088] Preparation of 5-(2-hydroxyethyl)-2,3-dihydrobenzofuran (DBFEtOH)

[0089] DBFAcOMe (227 g residue from Example 1) was dissolved in t-BuOH (600 ml) and $NaBH_4$ (46.8 g; 1.23 mol) was added. The resulting suspension was warmed to reflux and methanol (100 ml) was added very slowly in about 6 hrs

followed by maintaining the reaction mixture at reflux. After Methanol addition, the reaction was maintained at reflux for half an hour (IPC revealed complete ester transformation). 400 ml of t-BuOH-MeOH mixture was distilled off at atmospheric pressure. Water (400 ml) was added to residue and distillation was continued till complete solvent elimination. The reaction mixture was cooled to 70-75° C. and Toluene (300 ml) was added. Separated organic phase was washed with water (100 ml) and NaCl 15% (100 ml).

[0090] After solvent elimination under vacuum distillation an oily residue (176.8 g; 1.076 mol) was obtained. The residue solidified upon cooling.).

Example 3

[0091] Preparation of 5-(2-chloroethyl)-2,3-dihydrobenzofuran (DBF-EtCl) of formula II

[0092] $SOCl_2$ (74.7 g; 0.63 mol) was added to a solution of DBF-EtOH (80 g; 0.48 mol) in toluene (400 mL) maintaining the temperature below 25° C. The reaction mixture was stirred at 60° C. for 14 h and then cooled to room temperature.

[0093] The mixture pH was adjusted to 10-11 by addition of 10% NaOH (about 480 ml) while maintaining T<30° C. The organic phase was separated. Aqueous phase was extracted with Toluene (50 ml). Collected organic phases were washed twice with H_2O (100 mL each) and anhydrified by vacuum distillation. 20 g of Tonsil and 4,2 g of charcoal were added to the organic phase, and stirred for 30 min at room temperature, filtered off and washed with toluene (2×30 ml). The decolorized solution was concentrated under vacuum to eliminate toluene. The residue was dissolved in methanol (373 ml) and charcoal (2 g) was added. After 20 min at 50-55° C. charcoal was filtered off and washed with hot methanol (2×10 ml). Decolorized solution was cooled at 20-30° C. and DBF-EtCl crystallized. To the suspension was added at 25-30° C. in about 60 min, water (280 ml) obtaining a sticky but stirrable suspension. After 1 hr at 20-25° C. solid was filtered and washed three times with MeOH-Water 1:1 (20 ml each). Wet solid was dried at 35-40° C. for 15 hrs. Dry weight 81.8 g (0.45mol). Yield 92%).

Example 4

Preparation of Darifenacin Hydrobromide

[0094] Water (203 ml) Potassium carbonate (65 g) and DBF-EtCl (29.7 g) were heated to 60-65° C. To the mixture 3-(S)-(1-carbamoyl-1,1-diphenylmethyl)pyrrolidine tartrate ((S)-DIPAMP Tartrate) (65 g) was added and the heterogeneous mixture was heated to reflux (101-102° C.) for 5 hrs. After cooling to 85-90° C., n-Butanol (325 ml) was added and after stirring phases were separated.

[0095] The organic phase was washed twice with water (160 ml each) and then water was removed from organic phase by vacuum distillation. N-Butanol (160 ml) and acetic anhydride (3.25 ml) were added and the solution was stirred at 20-30° C. for 1 hr.

[0096] 48% HBr (g 25.5) was added drop-wise and the water was removed by vacuum distillation and DRF. HBr crystallised. Initial volume was restored by addition of n-BuOH. Suspension was stirred at 15-20° C. for 2 hrs, than product was recovered by filtration. The cake was washed with n-Butanol (3×30 ml) and wet solid (85-90 g) was crystallized without drying to obtain crude wet darifenacin hydrobromide.

[0097] The crude wet darifenacin hydrobromide (85 g), n-Butanol(455 ml) and charcoal (4.63 g) were warmed to reflux to obtain a solution. After half an hour charcoal was filtered off keeping mixture at near reflux. The clear solution at 100° C. was seeded with darifenacin hydrobromide and after 30 min at 100° C. the solution was cooled to 15-20° C. in 2 hrs. The suspension was stirred at 15-20° C. for 2 hrs and then the darifenacin hydrobromide was recovered by filtration. The filer cake was washed with n-butanol (3×25 ml) to give wet purified darifenacin hydrobromide. The wet purified darifenacin hydrobromide was dried under vacuum at 50-55° C. for 10-12 hrs. Dry weight 59.2 g. Overall Yield 77.2%).

Example 5

[0098] Synthesis of 5-(2-chloroethyl)-2,3-benzofuran (BF-EtCl) of Formula I

[0099] In a 500 ml reactor DBF-EtCl (30 g); NBS [N-Bromosuccinimide] freshly crystallized (29.3 g) Dibenzoylperoxide (0.67 g) and CC14 (210 ml) were loaded. Suspension was heated at gentle reflux (76° C.) for two hrs: vigorous gas evolution was observed. After two hrs reaction mixture was cooled and treated with $Na_2S_2O_5$ 15% (150 ml). After phase separation and washings solvent was eliminated by under vacuum distillation obtaining a red oil (35 g). 20 g of oil were purified by silica gel column chromatography (eluent: hexane). Fractions with HPLC purity higher then 85% were collected and after solvent elimination 8.64 g of BF-EtCl were isolated, HPLC purity 87.7%.).

Example 6

Preparation of (S)-Darifenacin Hydrobromide

[0100] A 50 ml reactor was loaded with 3-(S)-(+)-(1-carbamoyl- 1,1-diphenylmethyl)pyrrolidine Tartrate (4 g, 9.29 mmoles), 5-(2-chloroethyl)-2,3-dihydrobenzofuran (1.95 g, 10.68 mmoles), potassium carbonate (6.14 g, 44.42 mmoles), and water (12.5 ml), to obtain a heterogeneous mixture. The heterogeneous mixture was heated to reflux (103° C.) for 2.5 hours. After cooling dichloromethane, EtOAc or BuOAc (15 ml) were added, and, after stirring, the phases were separated. Acetic anhydride (0.5 ml) was added to the organic phase, and, after 1 hour at room temperature, the residual 3-(S)-(+)-(1-carbamoyl-1,1-diphenylmethyl)pyrrolidine was transformed into N-Acetyl derivative. The solvent was removed by distillation, and n-butanol (25 ml) was added to the residue. 48% hydrobromic acid (1.72 g) was also added, and the residual DCM was removed under vacuum distillation. In the case of EtOAc or BuOAc, distillation under vacuum is useful to eliminate water. Darifenacin hydrobromide crystallized, and, after cooling to room temperature, it was filtered and washed. (Wet solid 4.17 g).

Example 7

[0101] Preparing Darifenacin-HBr from 5-(2-chloroethyl)-2,3-dihydrobenzofaran of Formula II

[0102] The process of Example 6 was repeated to obtain crude darfienacin hydrobromide, and the crude darifeancin hydrobromide was recrystallized from n-butanol to obtain purified darifenacin hydrobromide. The purity of the purified darifenacin hydrobromide thus obtained was then analyzed. The results are summarized in the table below.

Tri-al	Components of Product	Level of oxidized impurity (area % by HPLC) 5-(2-chloroethyl)-2,3-benzofuran	Level of oxidized impurity (w/w % by HPLC) Oxidized darifenacin
1	5-(2-chloroethyl)-2,3-dihydrobenzofuran of formula II	0.40	N/A
	darifenacin hydrobromide (crude)	N/A	0.12
	darifenacin hydrobromide (purified)	N/A	0.12
2	5-(2-chloroethyl)-2,3-dihydrobenzofuran of formula II	0.50	N/A
	darifenacin hydrobromide (crude)	N/A	0.19
	darifenacin hydrobromide (purified)	N/A	0.20
3	5-(2-chloroethyl)-2,3-dihydrobenzofuran of formula II	0.16	N/A
	darifenacin hydrobromide (crude)	N/A	0.06
4	5-(2-chloroethyl)-2,3-dihydrobenzofuran of formula II	0.16	N/A
	darifenacin hydrobromide (crude)	N/A	0.06
	darifenacin hydrobromide (purified)	N/A	0.07
5	5-(2-chloroethyl)-2,3-dihydrobenzofuran of formula II	0.19	N/A
	darifenacin hydrobromide (crude)	N/A	0.09

* N/A = not applicable

Example 8

[0103] Preparing 5-(2-chloroethyl)-2,3-dihydrobenzofuran of Formula II in DMF, Toluene and in a Mixture of Dimethylformamide (DMF) and Toluene

Solvent	Temperature (° C.)	Level of 2-(benzofuran-5-yl)ethanol of formula V (area % by HPLC)	Level of 5-(2-chloroethyl)-2,3-benzofuran of formula I (area % by HPLC)
DMF	20° C.	nd	0.3
DMF	0-5° C.	nd	0.44
Toluene/ DMF 99:1	55° C.	0.01	1.15
Toluene	60° C.	0.03	0.21
Toluene	60° C.	nd	0.19
Toluene	60° C.	nd	0.14

* nd = not determined

Example 9

Correlation Between the Levels of the Oxidized Impurities in the Intermediates for Preparing Darifenacin Hydrobromide to the Level of Oxidized Darifenacin

[0104]

Level of impurity (area % by HPLC) BF-AcOH	Level of impurity (area % by HPLC) BF-AcOMe	Level of impurity (area % by HPLC) BF-EtOH	Level of impurity (area % by HPLC) BF-EtCl	Level of impurity (w/w % by HPLC) Oxidized darifenacin
0.18	0.17	0.16	0.18	0.09
0.50	0.53	0.47	0.30	0.49

* BF-AcOH is benzofuran-5-acetic acid; BF-AcOMe is benzofuran-5-methylester acetic acid; BF-EtOH is benzofuran-ethanol; and BF-EtCl is 5-(2-chloroethyl)-benzofuran.

Example 10

Analysis of ENABLEX® Tablet

[0105] A commercially available tablet of ENABLEX® was analyzed by the HPLC method described above for analysis of darifenacin HBr. A sample of ENABLEX® was prepared by crushing a tablet of ENABLEX® into a powder, adding 7 ml of methanol to the powder to form a suspension, and sonicating the suspension for 10 minutes. The resulting solution was filtered and 0.5 ml of the filtered solution was diluted with 0.5 ml of a 1:1 mixture of buffer (pH 7) and acetonitrile. The sample was then analyzed according to the HPLC method described above.

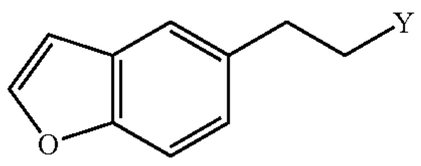
[0106] The analysis revealed that the ENABLEX® tablet contains a total amount of impurities of 1.07% area by HPLC, which distributes as follows: 0.03% area by HPLC of 3-(S)-(1-carbamoyl- 1,1-diphenylmethyl)pyrrolidine, 0.12% area by HPLC of oxidized darifenacin, and unidentified impurities in a total amount of 0.15% area by HPLC.

[0107] While it is apparent that the invention disclosed herein is well calculated to fulfill the objects stated above, it will be appreciated that numerous modifications and embodiments may be devised by those skilled in the art. Therefore, it is intended that the appended claims cover all such modifications and embodiments as falling within the true spirit and scope of the present invention.

We claim:

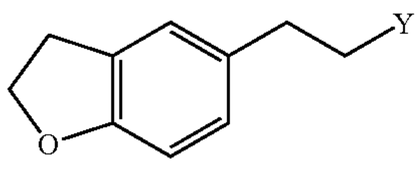
1. An HPLC method for determining the presence and level of oxidized darifenacin and salts thereof in a darifenacin hydrobromide sample comprising: (a) combining a darifenacin hydrobromide sample with a mixture of acetonitrile:water in a ratio of about 1:1, to obtain a solution; (b) injecting the solution into a 150×4.6 mm×5.0 μm Phenyl C6 column; (c) eluting the sample from the column using a mixture of acetonitrile:water in a ratio of 9:1 and buffer as an eluent; and (d) measuring the oxidized darifenacin content in the sample with a UV detector.

2. A process of determining the presence of a compound of the following formula I



I

in a sample comprising the compound of formula I and a compound of the following formula II



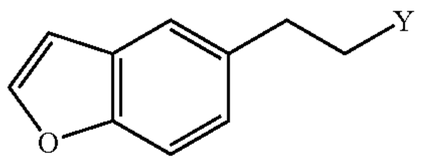
II

by a process comprising carrying out HPLC or TLC with the compound of formula I as a reference marker, wherein Y is a leaving group selected from the group consisting of Cl, I, brosyl, mesyl, tosyl, trifluoroacetyl, and trifluoromethansulfonyl.

3. The process of claim **2**, comprising: (a) measuring by HPLC or TLC the relative retention time corresponding to the compound of formula I in a reference marker sample; (b) determining by HPLC or TLC the relative retention time corresponding to the compound of formula I in a sample comprising a compound of formula I and a compound of formula II; and (c) determining the relative retention time of the compound of formula I in the sample by comparing the relative retention time of step (a) to the relative retention time of step (b).

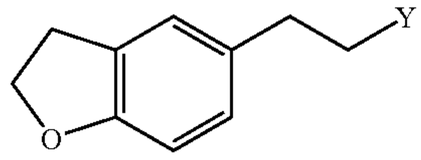
4. The process of claim **3**, wherein Y is Cl.

5. A process of determining the amount of a compound of the following formula I



I

in a sample comprising the compound of formula I and a compound of the following formula II



II

by a process comprising carrying out an HPLC with the compound of formula I as a reference standard, wherein Y is a leaving group selected from the group consisting of Cl, I, brosyl, mesyl, tosyl, trifluoroacetyl, and trifluoromethansulfonyl.

6. The process of claim **5**, comprising: (a) measuring by HPLC the area under a peak corresponding to a compound of formula I in a reference standard comprising a known amount of the compound of formula I; (b) measuring by HPLC the area under a peak corresponding to a compound of formula I in a sample comprising a compound of formula I and a compound of formula II; and (c) determining the amount of the compound of formula I in the sample by comparing the area of step (a) to the area of step (b).

7. The process of claim **6**, wherein Y is Cl.

8. The HPLC method of claim **3**, comprising: (a) combining a sample of the compound of formula I with a mixture of acetonitrile:water in a ration of about 1:1, to obtain a solution; (b) injecting the solution into a 250×4.6 mm×0.5 μm C18 column; (c) eluting the sample from the column using a mixture of acetonitrile and buffer as an eluent; and (d) measuring the amount of the compound of formula I in the sample with a UV detector at a wavelength of 215 nm.

9. the method of claim **8**, wherein Y is Cl.

10. The HPLC method of claim **6**, comprising: (a) combining a sample of the compound of formula I with a mixture of acetonitrile:water in a ratio of about 1:1, to obtain a solution; (b) injecting the solution into a 250×4.6 mm×0.5 μm C18 column; (c) eluting the sample from the column using a mixture of acetonitrile and buffer as an eluent; and (d) measuring the amount of the compound of formula I in the sample with a UV detector at a wavelength of 215 nm.

11. The method of claim **10**, wherein Y is Cl.

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