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(54) Titre : PURIFICATION D'ANALOGUES DE GLP-1
(54) Title: PURIFICATION OF GLP-1 ANALOGUES

(57) Abrégé/Abstract:
The present invention provides for purification of liraglutide using selective ion-pairing agents in the reversed phase-high performance liquid Chromatography, for purifying crude liraglutide from closely related impurities.

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(54) Title: PURIFICATION OF GLP-1 ANALOGUES

(57) Abstract: The present invention provides for purification of liraglutide using selective ion-pairing agents in the reversed phase-high performance liquid Chromatography, for purifying crude liraglutide from closely related impurities.

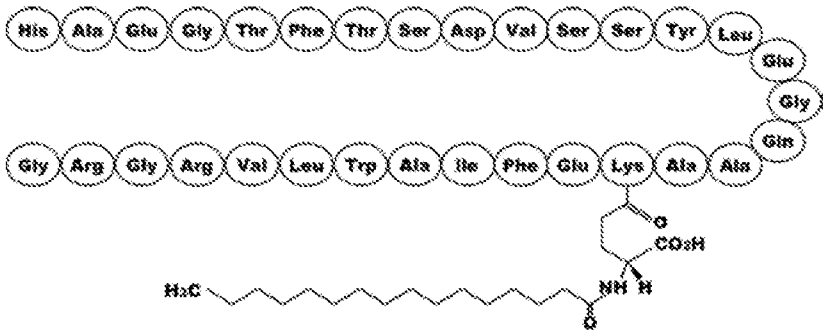
PURIFICATION OF GLP-1 ANALOGUES

RELATED APPLICATION

This application claims the benefit of priority of our Indian patent applications IN 201941004693 filed on February 06, 2019 which is incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to a method for purifying crude GLP-1 analogue, Liraglutide in particular which is represented by the **Formula-I.**



Formula-I

BACKGROUND

Liraglutide (VICTOZA®) is a glucagon-like peptide-1 (GLP-1) receptor agonist indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

Liraglutide, is a long acting analogue of the naturally occurring human glucagon like peptide-1 (GLP-1(7-37)) in which lysine at position 34 has been replaced with arginine and palmitoyl group has been attached via glutamoyl spacer to lysine at position 26.

Liraglutide (VICTOZA®), developed by Novo Nordisk got initial approval in United States in 2010 as subcutaneous injection.

Liraglutide due to its long peptide chain and high hydrophobicity due to palmitoyl group is highly difficult to purify.

Several attempts for purification of GLP-1 analogues including Liraglutide have been reported in the past.

Journal of Medicinal Chemistry 43, 1664-1669, 2000 discloses a purification process of Liraglutide by reversed phase-high performance liquid Chromatography (RP HPLC)using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitrile/TFA system.

The method as disclosed above results in a reduced purification yield of 35%.

WO2013117135 discloses a purification process of Liraglutide by RP HPLC using Isopropyl alcohol/TFA system.

The method as disclosed involves multiple purification steps involving 3 RP HPLC operations, which is a laborious process.

GLP-1 peptides are produced either by synthetic or by recombinant approach often have closely related impurities that are difficult to separate on RP-HPLC. These impurities are either isomeric or deletion/addition based impurities that have similar characteristics like the parent molecule. These closely related impurities pose a challenge in purification.

It is well-known that the use of RP-HPLC is limited for the separation and identification of complex mixtures having components with large variation in pKa values. Thus resolution of closely eluting impurities has always been challenging in chromatographic purification. In the resolution of organic ions with conventional HPLC methods, use of ion pair reagents can enhance peak shape and retention time when common remedies such as modifying eluent ratios or changing stationary phase fail. Thistechnique is sometimes referred as Ion pair chromatography (IPC).

IPC is a type of RP-HPLC in which ion pair reagents are added to the mobile phase to promote the formation of ion pairs with charged analytes, which makes the reversed phase column suitable for the separation of ionic molecules. The retention/separation follows a dynamic combination of reversed phase and ion pair-ion exchange mechanisms.

Ion pair reagents are comprised of a long linear alkyl chain (from C3 up to C16) and an ionic group which can reversibly adsorb to the alkyl chains (C8 or C18) of the RP-phase, forming a dynamic ion-exchanger, at which ionic compounds can be separated. There are

two main types of ion pair reagents, anionic alkyl sulfonates for basic compounds and cationic quaternary amines for acidic compounds. Commonly used Alkyl sulfonates in IPC are 1-hexanesulfonic acid sodium salt, 1-heptasulfonic acid sodium salt, 1-octanesulfonic acid sodium salt and the like. The selectivity of the system strongly depends on the choice and amount of the ion pair former in the mobile phase. Reagents with long chain lengths will considerably be better adsorbed onto the RP phase, affecting the retention in a positive way.

Separation of liraglutide from these closely related impurities was studied on RP-HPLC in absence and presence of ion pairing agents viz. sodium salts of 1-octane sulfonic acid, hexane sulfonic acid. It was observed that the resolution of closely related impurities was more effective in presence of the ion-pairing agent resulting in overall better purity as compared to the purification run, wherein ion-pairing agent was not used.

The present invention provides a method for purification of liraglutide from these closely related impurities was studied on RP-HPLC in absence and presence of ion pairing agents viz. sodium salts of propane sulfonic acid, butane sulfonic acid, pentane sulfonic acid, hexane sulfonic acid, heptane sulfonic acid, octane sulfonic acid, nonane sulfonic acid, decane sulfonic acid, undecane sulfonic acid, dodecane sulfonic acid, tridecane sulfonic acid.

It was observed that the resolution of closely related impurities was more effective in presence of the ion-pairing agent resulting in overall better purity as compared to the RP HPLC purification, wherein ion-pairing agent was not used.

SUMMARY OF THE INVENTION

Aspects of the present application provides processes for purification of liraglutide.

One aspect of the present invention discloses a method for purifying crude liraglutide, the method comprising:

a. obtaining a solution of liraglutide by dissolving crude liragluide in a mixture comprising an aqueous acid solution and acetonitrile;

b. subjecting the solution of crude liraglutide to a first HPLC purification using an aqueous acid solution and an ion pairing agent as mobile phase A and acetonitrile containing an alcohol as mobile phase B;
c. Subjecting the liraglutide from first HPLC purification to a second HPLC purification; and
d. Isolating the purified liraglutide.

Another aspect of the present invention discloses a method for purifying crude liraglutide, wherein ion-pairing agent is selected from salt of an alkane sulfonic acid.

Another aspect of the present invention discloses a method for purifying crude liraglutide, wherein the salt of an alkane sulfonic acid is selected from the group consisting of 1-octane sulfonic acid sodium salt or 1-heptane sulfonic acid sodium salt.

Another aspect of the present invention discloses a method for purifying crude liraglutide, wherein the salt of an alkane sulfonic acid is 1-hexane sulfonic acid sodium salt.

Another aspect of the present invention discloses a method for purifying crude liraglutide, wherein the aqueous acid solution is selected citric acid, acetic acid, trifluoroacetic acid or formic acid.

Another aspect of the present invention discloses a method for purifying crude liraglutide, by HPLC using ion pairing agent.

Instrumental parameters:

HPLC instrument parameters: Column : C8, 150 x 4.6 mm, 2.7µmColumn temperature : 60°CDetection : UVWavelength : 215 nm

ADVANTAGES OF PRESENT INVENTION

Crude liraglutide powder (Assay 20-25%; Purity 30-50%) is subjected to two sequential RP-HPLC purification steps under diverse conditions, followed by lyophilization to yield the pure Liraglutide. The present invention involves using selective ion-pairing agents in one of the RP-HPLC steps, for purifying crude liraglutide from closely related impurities. Absence of ion pairing agents in the process, results in no or poor resolution of the related impurities, leading to the impurities in the final API.

Comparison of Liraglutide purity profile without & with Octane-1-sulfonic acid as ion paring agent:

The below table provides a comparison of Liraglutide purity profile with respect to closely associated impurities present at RRT's 0.93, 0.98 & 1.06 without & with usageof Octane-1-sulfonic acid as ion paring agent.

STAGE	Ion Paring agent	AREA (%)			
		0.93 RRT	0.98 RRT	LIRAGLUTIDE	1.06 RRT
RP-HPLC-1 PURIFICATION	No Ion paring agent	0.84	0.31	92.55	0.4
	Octane-1-sulfonic acid sodium salt (OSA)	0.02	0.08	94.64	0

BRIEF DESCRIPTION OF THE FIGURES

In order that the disclosure may be readily understood and put into practical effect, reference will now be made to exemplary embodiments as illustrated with reference to the accompanying figures. The figures together with a detailed description below, are incorporated in and form part of the specification, and serve to further illustrate the embodiments and explain various principles and advantages, in accordance with the present disclosure wherein:

Figure 1: Illustrates the HPLC pattern of crude Liraglutide of **Formula I**.

Figure 2: Illustrates the HPLC pattern of Liraglutide of **Formula I** after purification without usage of OSA.

Figure 3: Illustrates the HPLC pattern of Liraglutide of **Formula I** after purification with usage of OSA.

Figure 4: Illustrates the HPLC pattern of Liraglutide of **Formula I** after purification with usage of HSA.

DETAILED DESCRIPTION OF THE INVENTION

The embodiments of the present invention are further described using specific examples herein after. The examples are provided for better understanding of certain embodiments of the invention and not, in any manner, to limit the scope thereof. Possible modifications

and equivalents apparent to those skilled in the art using the teachings of the present description and the general art in the field of the invention shall also form the part of this specification and are intended to be included within the scope of it.

EXAMPLES:

Example 1:

275.5 mg of crude Liraglutide, obtained from solid-phase synthesis was dissolved in 250 mM Citric acid monohydrate containing 10% Acetonitrile (v/v) filtered and subjected to a two-step RP-HPLC purification.

RP-HPLC-1:The crude liraglutide solution was loaded onto a 20 ml of C8 –substituted silica column (particle size 10-13 µm) equilibrated with about 60 ml of 100mM citric acid containing 0.05% w/v octane-1-sulphonic acid sodium salt (Mobile Phase A), 25% Acetonitrile: Isopropanol (7:3) (Mobile Phase B) pH 2.0. Post loading the column was washed with 8:2 Buffer A: Buffer B. Product was eluted by applying a gradient upto 60% B. Detection wavelength was kept at 215 nm. The chromatographic temperature was kept at 25°C. Fractions with purity of >91% were pooled and the average pool purity at RP-1 was >95% with closely related impurities less than 0.50%.

The liraglutide product from RP-HPLC-1 was taken further for RP-HLC-2.

Example 2:

275.5 mg of crude Liraglutide, obtained from solid-phase synthesis was dissolved in 250 mM Citric acid monohydrate containing 10% Acetonitrile (v/v) filtered and subjected to a two-step RP-HPLC purification.

RP-HPLC-1:

The crude liraglutide solution was loaded onto a 20 ml of C8 –substituted silica column (particle size 10-13 µm) equilibrated with about 60 ml of 100mM citric acid containing 0.05% 1- hexane sulphonic acid sodium salt (Mobile Phase A), 25% Acetonitrile: Isopropanol (7:3) (Mobile Phase B) pH 2.0. Post loading the column was washed with 8:2 Buffer A: Buffer B. Product was eluted by applying a gradient upto 60%. Detection wavelength was kept at 215 nm. The chromatographic temperature was kept at 25°C.

Fractions were collected and analyzed for purity. Fractions with purity of >91% were pooled.

The purity of the RP-HPLC-1 purified liraglutide HPLC chromatogram is as shown in **Figure 4.**

HPLC purity of the RP-HPLC-1 purified liraglutide was >95% with closely related impurities less than 2.0%

pH of these pool was adjusted to 7.8 and distilled at 35°C to remove organic solvent. Precipitation was done at pH-4.9 and RP-HPLC-1 purified liraglutide was isolated.

The liraglutide product from RP-HPLC-1 was taken further for RP-HLC-2.

Example 3:

275.5 mg of crude Liraglutide, obtained from solid-phase synthesis was dissolved in 250 mM Citric acid monohydrate containing 10% Acetonitrile (v/v) filtered and subjected to a two-step RP-HPLC purification.

RP-HPLC-1:

The crude liraglutide solution was loaded onto a 20 ml of C8 –substituted silica column (particle size 10-13 µm) equilibrated with about 60 ml of 100mM citric acid (Mobile Phase A), 25% Acetonitrile: Isopropanol (7:3) (Mobile Phase B) pH 2.0. Post loading the column was washed with (8:2) Buffer A: Buffer B. Product was eluted by applying a gradient upto 60% B. Detection wavelength was kept at 215 nm. The chromatographic temperature was kept at 25°C. Fractions were collected and analyzed for purity. Fractions with purity of >91% were pooled.

HPLC purity of the RP-HPLC-1 purified liraglutide was >95% with closely related impurities less than 2.0%.

The purity of the RP-HPLC-1 purified liraglutide HPLC chromatogram is as shown in **Figure 2.**

The liraglutide product from RP-HPLC-1 was taken further for RP-HLC-2.

Example 4:

32.6 g of crude Liraglutide, obtained from solid-phase synthesis was dissolved in 250 mM Citric acid monohydrate containing 10% Acetonitrile (v/v) filtered and subjected to a two-step RP-HPLC purification.

RP-HPLC-1:

The crude liraglutide solution was loaded onto a 2.4L of C8 –substituted silica column (particle size 10-13 µm) equilibrated with about 7.2L of 100mM citric acid containing 0.05% octane-1-sulphonic acid sodium salt (Mobile Phase A), 25% Acetonitrile: Isopropanol (7:3) (Mobile Phase B) pH 2.0. Post loading the column was washed with 8:2 Buffer A: Buffer B. Product was eluted by applying a gradient upto 60% B. Detection wavelength was kept at 215 nm. The chromatographic temperature was kept at 25°C. Fractions were collected and analyzed for purity. Fractions with purity of >91% were pooled. The purity of the RP-HPLC-1 purified liraglutide HPLC chromatogram is as shown in **Figure 3.**

HPLC purity of the RP-HPLC-1 purified liraglutide was >94% with closely related impurities less than 0.50%.

pH of these pool was adjusted to 7.8 and distilled at 35°C to remove organic solvent. Precipitation was done at pH-4.9 and RP-HPLC-1 purified liraglutide was isolated.

The liraglutide product from RP-HPLC-1 was taken further for RP-HLC-2.

RP-HPLC-2:

3.1L of the RP-HPLC-1 purified liraglutide dissolved in 50 mM Di-Sodium hydrogen phosphate containing 25% Methanol at 3 mg/ml was loaded onto a a 2.4 L of C8 – substituted silica column (particle size 10-13 µm) equilibrated with about 7.2 L of 50mM Sodium phosphate buffer pH 7.5 containing 5% Acetonitrile. Product was eluted by applying a gradient upto 41% B. Detection wavelength was kept at 215 nm. The chromatographic temperature was kept at 25°C. Individual fractions were collected and analyzed for purity. Fractions with purity of >98.0% were pooled and distilled at 35°C to remove organic solvent. Precipitation was done at pH 4.9. The purified Liraglutide was subjected to lyophilization. HPLC purity of the lyophilized powder was >99% with no impurity more than 0.20%.

CLAIMS:

1. A method for purifying crude liraglutide, the method comprising:
   a. obtaining a solution of liraglutide by dissolving crude liragluide in a mixture comprising an aqueous acid solution and acetonitrile;
   b. subjecting the solution of crude liraglutide to a first HPLC purification using an aqueous acid solution and an ion pairing agent as mobile phase A and acetonitrile containing an alcohol as mobile phase B;
   c. Subjecting the liraglutide from first HPLC purification to a second HPLC purification; and
   d. Isolating the purified liraglutide.

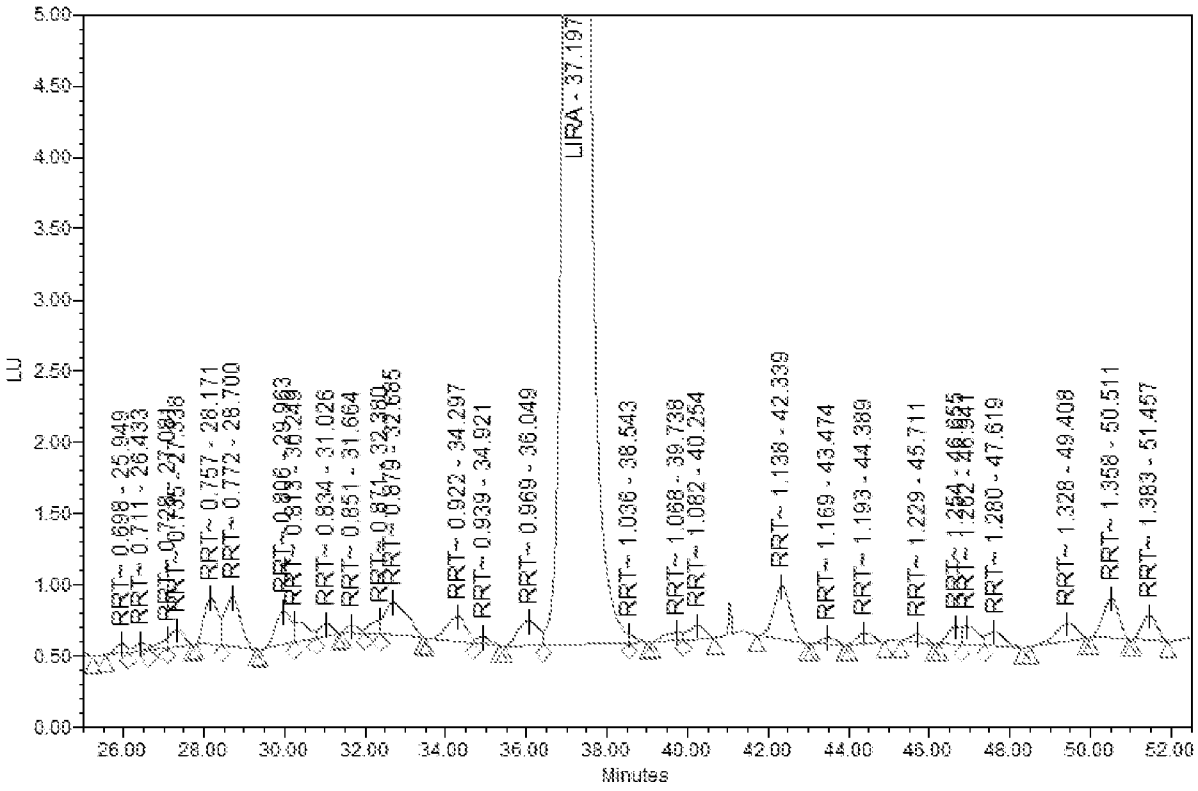
2. The method of claim 1, wherein ion-pairing agent is selected from salt of an alkane sulfonic acid.

3. The method of claim 2, wherein the salt of an alkane sulfonic acid is selected from the group consisting of 1-octane sulfonic acid sodium salt or 1-hepane sulfonic acid sodium salt or 1-hexane sulfonic acid sodium salt.

4. The method of claim 2, wherein the salt of an alkane sulfonic acid is 1-octane sulfonic acid sodium salt.

5. The method of claim 1, wherein the aqueous acid solution is selected citric acid, acetic acid, trifluoroacetic acid or formic acid.

6. A method for purification of liraglutide by HPLC using ion pairing agent.


LU
Minutes
LIRA - 37.197
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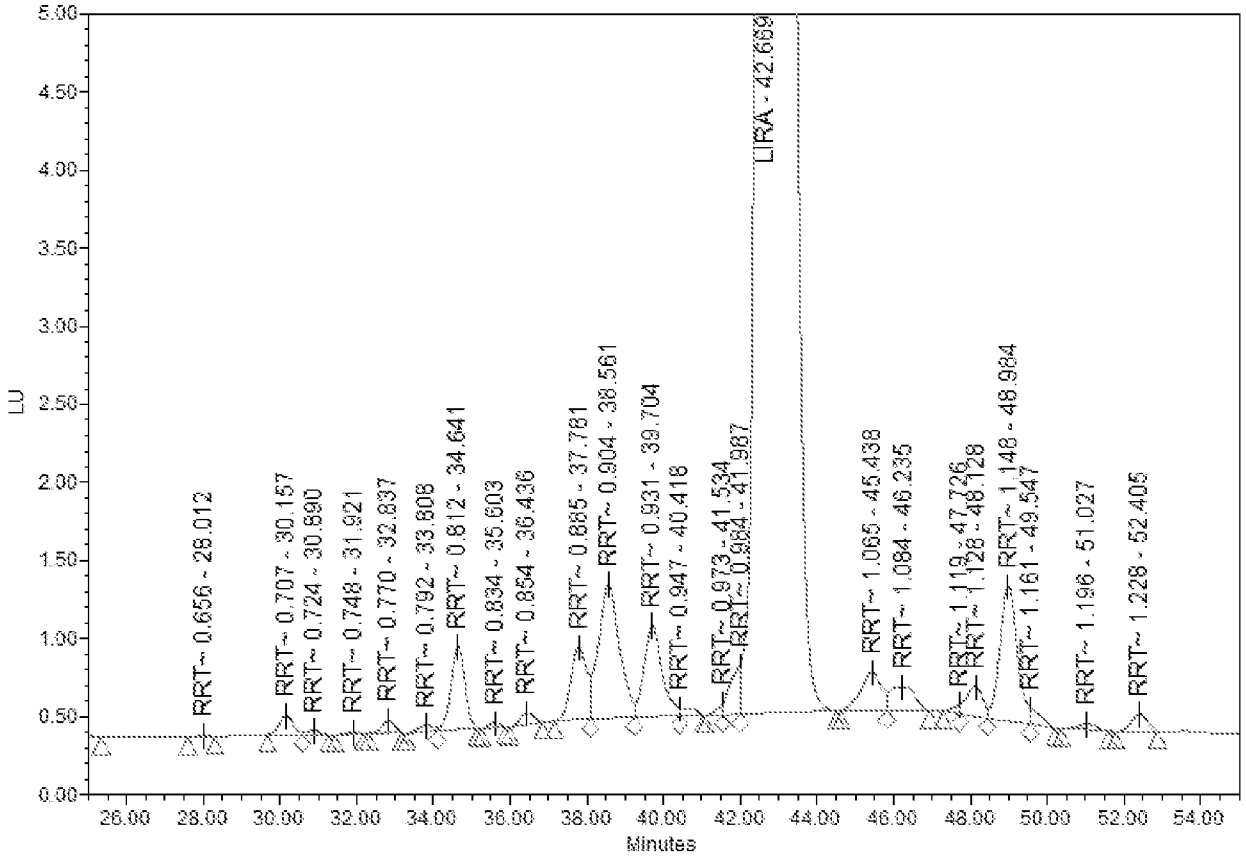
RRT~ 0.711 - 26.433
RRT~ 0.757 - 28.171
RRT~ 0.772 - 28.700
RRT~ 0.834 - 31.026
RRT~ 0.851 - 31.664
RRT~ 0.922 - 34.297
RRT~ 0.939 - 34.921
RRT~ 0.969 - 36.049
RRT~ 1.036 - 38.543
RRT~ 1.068 - 39.738
RRT~ 1.082 - 40.254
RRT~ 1.138 - 42.339
RRT~ 1.169 - 43.474
RRT~ 1.193 - 44.369
RRT~ 1.229 - 45.711
RRT~ 1.280 - 47.619
RRT~ 1.328 - 49.408
RRT~ 1.358 - 50.511
RRT~ 1.383 - 51.457

Figure 1


LU
Minutes
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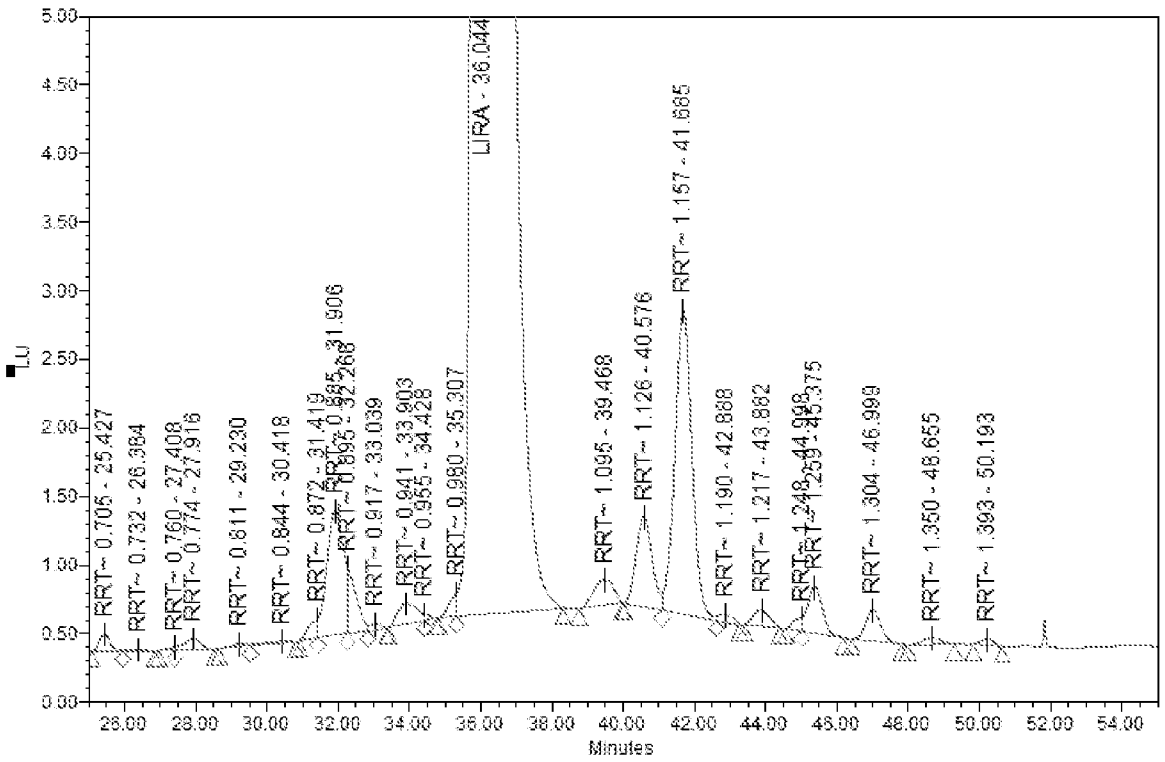
RRT~ 0.707 - 30.157
RRT~ 0.724 - 30.890
RRT~ 0.748 - 31.921
RRT~ 0.770 - 32.837
RRT~ 0.792 - 33.808
RRT~ 0.812 - 34.641
RRT~ 0.834 - 35.603
RRT~ 0.854 - 36.436
RRT~ 0.885 - 37.781
RRT~ 0.904 - 38.561
RRT~ 0.931 - 39.704
RRT~ 0.947 - 40.418
RRT~ 0.973 - 41.534
RRT~ 0.984 - 41.987
LIRA - 42.669
RRT~ 1.065 - 45.438
RRT~ 1.084 - 46.235
RRT~ 1.119 - 47.726
RRT~ 1.128 - 48.128
RRT~ 1.148 - 48.984
RRT~ 1.161 - 49.547
RRT~ 1.196 - 51.027
RRT~ 1.228 - 52.405

Figure 2

3/4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RRT~ 0.732 - 26.384
RRT~ 0.760 - 27.408
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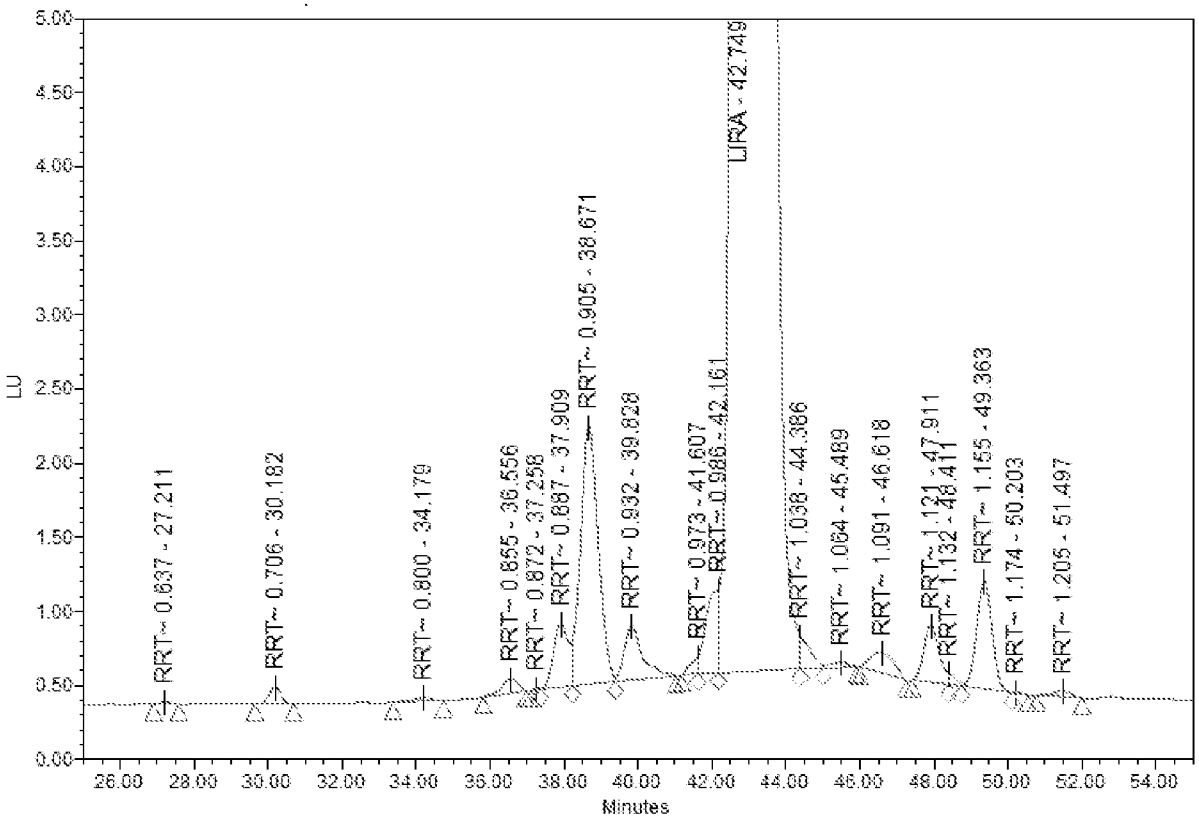
RRT~ 0.811 - 29.230
RRT~ 0.844 - 30.418
RRT~ 0.872 - 31.419
RRT~ 0.895 - 32.268
RRT~ 0.917 - 33.039
RRT~ 0.941 - 33.903
RRT~ 0.955 - 34.428
RRT~ 0.980 - 35.307
RRT~ 1.095 - 39.468
RRT~ 1.126 - 40.576
RRT~ 1.157 - 41.685
RRT~ 1.190 - 42.888
RRT~ 1.217 - 43.862
RRT~ 1.248 - 44.998
RRT~ 1.259 - 45.375
RRT~ 1.304 - 46.999
RRT~ 1.350 - 48.655
RRT~ 1.393 - 50.193
LU
Minutes

Figure 3


LU
Minutes
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RRT~ 0.706 - 30.182
RRT~ 0.800 - 34.179
RRT~ 0.855 - 36.556
RRT~ 0.872 - 37.258
RRT~ 0.887 - 37.909
RRT~ 0.905 - 38.671
RRT~ 0.932 - 39.828
RRT~ 0.973 - 41.607
RRT~ 0.986 - 42.161
LIRA - 42.749
RRT~ 1.036 - 44.386
RRT~ 1.064 - 45.489
RRT~ 1.091 - 46.618
RRT~ 1.121 - 47.911
RRT~ 1.132 - 48.411
RRT~ 1.155 - 49.363
RRT~ 1.174 - 50.203
RRT~ 1.205 - 51.497

Figure 4