The present invention relates to a new HPLC method for the analysis of the drug substance sunitinib and related substances. In particular, the present invention relates to a new HPLC method for the analysis of the salt sunitinib malate and related substances. In a first method the mobile phase comprises two or more liquids and the relative concentration of the liquids is varied to a predetermined gradient. In a second method the mobile phase comprises an alcohol. In a third method the mobile phase comprises acetic acid. A fourth method comprises the detection and optional quantification of 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrol-1-one and/or carboxamide. The present invention also relates to sunitinib and associated pharmaceutical compositions from which samples have been analysed by the methods of the invention and/ or which are substantially free of specific impurities.
HPLC METHOD FOR ANALYZING SUNITINIB

Field of the invention

The present invention relates to a new HPLC method for the analysis of the drug substance sunitinib and related substances. In particular, the present invention relates to a new HPLC method for the analysis of the salt sunitinib malate and related substances. In a first method the mobile phase comprises two or more liquids and the relative concentration of the liquids is varied to a predetermined gradient. In a second method the mobile phase comprises an alcohol. In a third method the mobile phase comprises acetic acid. A fourth method comprises the detection and optional quantification of 5-fluoro-2,3-dihydro-lH-indole-2-one and/or N-[2-(clietiylaniino)emyl]-2,4-dimethyl-5-formyl-lH-pyrrole-3-carboxamide. The present invention also relates to sunitinib and associated pharmaceutical compositions from which samples have been analysed by the methods of the invention and/or which are substantially free of specific impurities.

Background art

In order to secure marketing approval for a pharmaceutical product, a manufacturer must submit detailed evidence to the appropriate regulatory authorities to prove that the product is suitable for release onto the market. It is, therefore, necessary to satisfy regulatory authorities that the product is acceptable for administration to humans and that the particular pharmaceutical composition, which is to be marketed, is sufficiently free from impurities at the time of release and that it has acceptable storage stability (shelf life).

Therefore, applications to regulatory authorities for the approval of drug substances must include analytical data which demonstrate that impurities in the active pharmaceutical ingredient (API), at the time of manufacture and during storage, are absent or are present only in acceptable levels.

The likely impurities in APIs and pharmaceutical compositions include residual quantities of synthetic precursors (intermediates), by-products which arise during synthesis of the API, residual solvents, isomers of the API (e.g. geometrical isomers, diastereomers or
enantiomers), contaminants which are present in materials used in the synthesis of the API or in the preparation of the pharmaceutical composition, and unidentified adventitious substances. Other impurities which may appear on storage include degradants of the API, for instance formed by hydrolysis or oxidation.

The health authorities have very stringent standards and manufacturers must demonstrate that their product is relatively free from impurities or within acceptable limits and that these standards are reproducible for each batch of pharmaceutical product that is produced.

The tests that are required to demonstrate that the API or pharmaceutical compositions are safe and effective include purity assay, related substances, content uniformity and dissolution tests. The purity assay test determines the purity of the test product when compared to a standard of a known purity, while the related substances test is used to quantify all the impurities present in the product. The content uniformity test ensures that batches of product like a tablet contain a uniform amount of API and the dissolution test ensures that each batch of product has a consistent dissolution and release of the API.

The technique of choice for the analysis of APIs or pharmaceutical compositions (e.g. the tablet or capsule) is usually High Performance Liquid Chromatography (HPLC) coupled with a UV-Visible detector. The API and the impurities present, if any, are separated on the HPLC stationary phase and they can be detected and quantified using their response obtained from the UV-Visible detector.

HPLC is a chromatographic separation technique in which high-pressure pumps force the substance or mixture being analysed together with a mobile phase, also referred to as the eluent, through a separating column containing the stationary phase.

HPLC analysis may be performed in isocratic or gradient mode. In isocratic mode, the mobile phase composition is constant throughout. A gradient HPLC mode is carried out by a gradual change over a period of time in the percentage of the two or more solvents making up the mobile phase. The change in solvent is controlled by a mixer which mixes the solvents to produce the mobile phase prior to its passing through the column.
If a substance interacts strongly with the stationary phase, it remains in the column for a relatively long time, whereas a substance that does not interact with the stationary phase as strongly elutes out of the column sooner. Depending upon the strength of interactions, the various constituents of the analyte appear at the end of the separating column at different times, known as retention times, where they can be detected and quantified by means of a suitable detector, such as a UV-Visible detector.

Sunitinib, chemically known as N-[2-(diemylamiino)emyl]-5-[(Z)-(5-fluoro-1,2-dihydro-2-oxo-3H-indol-3-yUdine)memyl]-2,4-dimemyl-1H-pyrrole-3-carboxami de, is a multi-targeted receptor tyrosine kinase (RTK) inhibitor and its salt, sunitinib malate (I), specifically sunitinib (2S)-2-hydroxybutanedioate (I), is currently marketed with the brand name Sutent® as an oral anti cancer drug.

Several HPLC methods have been reported in the literature for the estimation of sunitinib malate in biological fluids and pharmaceutical formulations, but these methods have not been primarily developed for the detection and quantitation of all impurities in sunitinib malate (see, for example: S. Baratte et al, J. Chromatography A, vol. 1024 (1-2), pages 87-94, 2004; P. Minldn et al, J. Chromatography B, vol. 874 (1-2), pages 84-88, 2008; B. Blanchet et al, Clinica Chimica Acta, vol. 404 (2), pages 134-139, 2009).

However, the current HPLC methods are not particularly suitable for the detection and estimation of total impurities, especially with respect to unknown impurities that are present in a sunitinib or sunitinib malate sample, particularly samples synthesized by the process disclosed in patent US 6573293.
Therefore, the HPLC methods reported in the prior art are not particularly convenient or suitable for analysing sunitinib API, particularly with respect to related substances.

Consequently, although several HPLC methods have been reported in the prior art for the analysis of sunitinib and its impurities, there is still a need for an alternative method which avoids the problems associated with the known methods as discussed above.

Studies by the present inventors have culminated in the development and validation of a new, efficient, reproducible and simple HPLC method for the analysis of sunitinib, particularly with respect to the related substances formed during the synthetic process.

Object of the invention

It is, therefore, an object of the present invention to provide a new, accurate and sensitive HPLC method for the detection and quantitation of all intermediates and related substances that are formed and may remain in the batches of sunitinib whilst avoiding the typical problems associated with the prior art methods.

A particular object of the invention is to provide a new, accurate and sensitive HPLC method for the detection and quantitation of all intermediates and related substances that are formed and may remain in the batches of sunitinib or sunitinib malate synthesized by the process disclosed in patent US 6573293.

Summary of the invention

The term "sunitinib" as used herein throughout the description and claims means sunitinib and/or any salt, solvate, hydrate, anhydrate, tautomer or isomer thereof. The present invention is particularly useful for the analysis of sunitinib (S)-malate.

A first aspect of the present invention provides a HPLC method for analysing sunitinib or a salt thereof, wherein the mobile phase comprises two or more liquids and the relative concentration of the liquids is varied to a predetermined gradient.
Preferably the mobile phase comprises a first liquid A which is aqueous based, such as water or an aqueous solution of a buffer.

Preferably the buffer is selected from an acid, an organic salt, an inorganic salt, an organic base or a mixture thereof. More preferably the buffer is selected from an acid, an organic salt, an inorganic salt or a mixture thereof.

Typically, the buffer is a phosphate salt, an acetate salt, a trifluoroacetate salt, a formate salt, acetic acid, trifluoroacetic acid, formic acid, a phosphoric acid such as orthophosphoric acid, or a mixture thereof. More typically, the buffer is a phosphate salt, an acetate salt, a formate salt, acetic acid, trifluoroacetic acid, or a mixture thereof.

Where the buffer is a salt, preferably the counter cation is an ammonium cation.

Most preferably the buffer is a mixture of an acetate salt, such as ammonium acetate, and an acid, more preferably an organic acid, such as acetic acid.

The salt in the buffer can be present at a concentration of 0.001 to 0.2 M, preferably at a concentration of 0.005 to 0.1 M, more preferably at a concentration of 0.01 to 0.1 M, and most preferably at a concentration of about 0.05 M; and/or the acid can be present at a concentration of 0.001 to 0.2 % v/v, preferably at a concentration of 0.005 to 0.1 % v/v, more preferably at a concentration of 0.01 to 0.1 % v/v, and most preferably at a concentration of about 0.05 % v/v.

Preferably the buffer is a mixture of ammonium acetate present at a concentration of 0.01 to 0.1 M and acetic acid at a concentration of 0.01 to 0.1 % v/v. Most preferably the buffer is a mixture of ammonium acetate present at a concentration of about 0.05 M and acetic acid at a concentration of about 0.05 % v/v.

Preferably the pH of the buffer solution is approximately 2 to 6.5. More preferably the pH of the buffer solution is approximately 4 to 6. Most preferably the pH of the buffer solution is about 5.5.
Typically, the method of the first aspect of the present invention is carried out at a column temperature between approximately 15 to 40°C.

The mobile phase preferably comprises a second liquid B which is or comprises an organic solvent, preferably selected from an alkyl alcohol, such as methanol, ethanol, propanol or iso-propanol, or acetonitrile, or a mixture thereof.

In one embodiment of the first aspect of the present invention, the second liquid B comprises or is a polar protic organic solvent such as acetic acid, methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol, or a mixture thereof. Preferably the polar protic organic solvent is an alcohol such as a C₁-C₆ alcohol. More preferably the alcohol is an alkyl alcohol. More preferably still the alcohol is a C₁-C₄ alkyl alcohol such as methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol. Most preferably the second liquid B is methanol.

For the purposes of the present invention, an "alkyl" group is defined as a monovalent saturated hydrocarbon, which may be straight-chained or branched, or be or include cyclic groups. An alkyl group may optionally include one or more heteroatoms N, O or S in its carbon skeleton. Examples of alkyl groups are methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, n-pentyl and iso-pentyl groups. Preferably an alkyl group is straight-chained or branched and does not include any heteroatoms in its carbon skeleton. Preferably an alkyl group is a C₁-C₁₂ alkyl group, which is defined as an alkyl group containing from 1 to 12 carbon atoms. More preferably an alkyl group is a C₁-C₆ alkyl group, which is defined as an alkyl group containing from 1 to 6 carbon atoms.

In another embodiment of the first aspect of the present invention, the second liquid B is substantially water miscible.

As used herein, the term "substantially miscible" in relation to two liquids X and Y means that when mixed together at 20°C and 1 atmosphere pressure, X and Y form a single phase between two mole fractions of Y, x₁ and x₂, wherein the magnitude of Δχₗ = x₂ - x₁ is at least 0.05. For example, X and Y may form a single phase where the mole fraction of Y,
\( x_\gamma \), is from 0.40 to 0.45, or from 0.70 to 0.75; in both cases \( \Delta x_\gamma = 0.05 \). Preferably the magnitude of \( \Delta x_\gamma \) is at least 0.10, more preferably at least 0.25, more preferably at least 0.50, more preferably at least 0.75, more preferably at least 0.90, even more preferably at least 0.95. Most preferably the term "substantially miscible" in relation to two liquids X and Y means that when mixed together at 20°C and 1 atmosphere pressure, X and Y form a single phase when mixed together in any proportion.

In one embodiment of the first aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% acetonitrile by volume. In one embodiment, the mobile phase contains no acetonitrile.

In another embodiment of the first aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% of any organic dipolar aprotic solvent by volume. In one embodiment, the mobile phase contains no organic dipolar aprotic solvent.

A preferred embodiment of the first aspect of the present invention is when the first liquid A is an aqueous solution of a buffer comprising an acetate salt mixed with acetic acid, and the second liquid B is methanol.

A particularly preferred embodiment of the first aspect of the present invention is when the first liquid A is a mixture of 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol.

In one embodiment the method of the first aspect of the present invention comprises a gradient programming so that the relative concentration of the liquids A and B by volume is typically varied to a gradient between 100 % A : 0 % B to 0 % A : 100 % B over a period of 10 to 180 minutes. Preferably the gradient is between 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 120 minutes, more preferably 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 60 minutes.

Alternatively, the HPLC method of the first aspect of the present invention may comprise a gradient programming so that the relative concentration of the liquids A and B by volume
starts at a first ratio, then is varied to a first gradient over a first period of time, to arrive at
a second ratio, then optionally is maintained at said second ratio for a second period of
time.

The first ratio may be 70-90 % A : 10-30 % B. Preferably the first ratio is 75-85 % A : 15-
25 % B. Most preferably the first ratio is about 80 % A : 20 % B.

The first period of time may be from 0 to 120 minutes. Preferably the first period of time is
from 15 to 60 minutes. Most preferably the first period of time is about 30 minutes.

The second ratio may be 0-20 % A : 80-100 % B. Preferably the second ratio is 5-15 % A :
85-95 % B. Most preferably the second ratio is about 10 % A : 90 % B.

The second period of time may be from 0 to 60 minutes. Preferably the second period of
time is from 2 to 20 minutes. Most preferably the second period of time is about 5 minutes.

Preferably a mobile phase flow rate of between 0.01 and 10 ml/min is used, more
preferably a mobile phase flow rate of between 0.1 and 4 ml/min is used, more preferably
still a mobile phase flow rate of between 0.5 and 1.5 ml/min is used, most preferably a
mobile phase flow rate of about 1 ml/min is used.

In one embodiment of the first aspect of the present invention, the stationary phase used is
a gel, preferably a silica gel.

Preferably the stationary phase used in the first aspect of the present invention is reverse
phase such as octadecylsilyl silica gel, octylsilyl silica gel, phenylalkyl silica gel, cyanopropyl
silica gel, aminopropyl silica gel or an alkyl-diol silica gel. Particularly suitable stationary
phases include octadecylsilyl silica gel or octylsilyl silica gel. A particularly preferred
stationary phase comprises an Inertsil ODS 3V (250 mm x 4.6 mm), 5µm column.

Preferably the stationary phase has a particle size of between 0.1 and 100µm, or between
0.5 and 25µm, or between 1 and 10µm, or between 4.5 and 6µm. More preferably the
stationary phase has a particle size of about 5µm.
Preferably the stationary phase has a pore size of between 10 and 100Å, or between 25 and 500Å, or between 50 and 200Å. More preferably the stationary phase has a pore size of between 75 and 125Å, or between 90 and 110Å. Most preferably the stationary phase has a pore size of about 100Å.

In one embodiment of the first aspect of the present invention, the chromatography is carried out in a column between 10mm and 5000mm in length, or in a column between 50mm and 1000mm in length, or between 100mm and 500mm in length. More preferably the chromatography is carried out in a column between 200mm and 280mm in length. Most preferably the chromatography is carried out in a column about 250mm in length.

The chromatography may be carried out in a column between 0.01mm and 100mm in internal diameter, or between 0.1mm and 50mm in internal diameter, or between 1mm and 10mm in internal diameter. More preferably the chromatography is carried out in a column about 4.6mm in internal diameter.

A particularly preferred method according to the first aspect of the present invention is when the first liquid A is 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol and the gradient is as follows:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A (by volume)</th>
<th>% B (by volume)</th>
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<tbody>
<tr>
<td>0</td>
<td>80</td>
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<tr>
<td>45</td>
<td>80</td>
<td>20</td>
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</tbody>
</table>

The eluent may be analysed by a detector such as a UV and/ or visible spectrophotometer, a fluorescence spectrophotometer, a differential refractometer, an electrochemical detector, a mass spectrometer, a light scattering detector or a radioactivity detector.
In one embodiment of the first aspect of the present invention the HPLC method detects and optionally quantifies in a single run one or more impurities selected from:
5-fluoro-2,3-dihydro-1H-indole-2-one; and
N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

Preferably the HPLC method detects and optionally quantifies in a single run both 5-fluoro-2,3-dihydro-1H-indole-2-one and N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

In a preferred embodiment the HPLC method according to the first aspect of the present invention efficiently detects and quantifies in a single run all impurities including those selected from the following compounds:
5-fluoro-2,3-dihydro-1H-indole-2-one; and
N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

In one embodiment of the first aspect of the present invention, 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide is used as internal or external reference marker, or as internal or external reference standard.

In another embodiment of the first aspect of the present invention, sunitinib, clozapine and/or ranitidine is used as internal or external reference marker, or as internal or external reference standard.

In one embodiment of the first aspect of the present invention, the sunitinib is in the form of a salt, solvate, hydrate or anhydrate. Preferably the method of the first aspect of the present invention is for analysing sunitinib malate. More preferably the method of the first aspect of the present invention is for analysing sunitinib (S)-malate. Optionally, the method of the first aspect of the present invention is for analysing anhydrous sunitinib, such as anhydrous sunitinib free base or anhydrous sunitinib (S)-malate.
In another embodiment of the first aspect of the present invention, the HPLC method is used for the analysis of sunitinib or a salt thereof that is suitable for use in a pharmaceutical composition.

Preferably the HPLC method is used for the analysis of sunitinib or a salt thereof that has not entered the human or animal body. Preferably the sunitinib or the salt thereof that is analysed is not in contact with a human or animal bodily fluid such as plasma. Preferably the sunitinib or the salt thereof that is analysed is not in contact with a human or animal bodily tissue such as liver, kidney, brain or white fat tissue. Preferably the sunitinib or the salt thereof that is analysed is not in solution.

In another embodiment of the first aspect of the present invention, the HPLC method is used for the analysis of a pharmaceutical composition comprising sunitinib or a salt thereof.

In yet another embodiment of the first aspect of the present invention, the HPLC method is used for the analysis of a substance comprising at least 5% sunitinib or a salt thereof by weight. Preferably the substance comprises at least 10%, at least 25%, at least 50%, at least 75% or at least 90% sunitinib or a salt thereof by weight. Most preferably the substance comprises at least 95% sunitinib or a salt thereof by weight.

In yet another embodiment of the first aspect of the present invention, the HPLC method is used for the analysis of a substance comprising sunitinib or a salt thereof as the only active pharmaceutical ingredient.

A second aspect of the present invention provides a chromatographic method for analysing sunitinib or a salt thereof, wherein the mobile phase comprises an alcohol.

Preferably the alcohol is substantially water miscible.

In one embodiment of the second aspect of the present invention, the alcohol is a C$_1$-C$_4$ alcohol. Preferably the alcohol is an alkyl alcohol. More preferably the alcohol is a C$_1$-C$_4$
alkyl alcohol such as methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol. Most preferably the alcohol is methanol.

In a preferred embodiment of the second aspect of the present invention, the mobile phase comprises two or more liquids, including a first liquid A and a second liquid B, wherein at least one of said liquids comprises the alcohol. Preferably the second liquid B is the alcohol.

Preferably the first liquid A is aqueous based, such as water or an aqueous solution of an acid, an organic salt, an inorganic salt, an organic base or a mixture thereof. More preferably the buffer is selected from an acid, an organic salt, an inorganic salt or a mixture thereof.

Typically, the buffer is a phosphate salt, an acetate salt, a trifluoroacetate salt, a formate salt, acetic acid, trifluoroacetic acid, formic acid, a phosphoric acid such as orthophosphoric acid, or a mixture thereof. More typically, the buffer is a phosphate salt, an acetate salt, a formate salt, acetic acid, trifluoroacetic acid, or a mixture thereof.

Where the buffer is a salt, preferably the counter cation is an ammonium cation.

Most preferably the buffer is a mixture of an acetate salt, such as ammonium acetate, and an acid, more preferably an organic acid, such as acetic acid.

The salt in the buffer can be present at a concentration of 0.001 to 0.2 M, preferably at a concentration of 0.005 to 0.1 M, more preferably at a concentration of 0.01 to 0.1 M, and most preferably at a concentration of about 0.05 M; and/or the acid can be present at a concentration of 0.001 to 0.2 % v/v, preferably at a concentration of 0.005 to 0.1 % v/v, more preferably at a concentration of 0.01 to 0.1 % v/v, and most preferably at a concentration of about 0.05 % v/v.

Preferably the buffer is a mixture of ammonium acetate present at a concentration of 0.01 to 0.1 M and acetic acid at a concentration of 0.01 to 0.1 % v/v. Most preferably the buffer is a mixture of ammonium acetate present at a concentration of about 0.05 M and acetic acid at a concentration of about 0.05 % v/v.
Preferably the pH of the buffer solution is approximately 2 to 6.5. More preferably the pH of the buffer solution is approximately 4 to 6. Most preferably the pH of the buffer solution is about 5.5.

In one embodiment of the second aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% acetonitrile by volume. In one embodiment, the mobile phase contains no acetonitrile.

In another embodiment of the second aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% of any organic dipolar aprotic solvent by volume. In one embodiment, the mobile phase contains no organic dipolar aprotic solvent.

A preferred embodiment of the second aspect of the present invention is when the first liquid A is an aqueous solution of a buffer comprising an acetate salt mixed with acetic acid, and the second liquid B is methanol.

A particularly preferred embodiment of the second aspect of the present invention is when the first liquid A is a mixture of 0.05 M ammonium acetate containing 0.05% v/v acetic acid and the second liquid B is methanol.

In one embodiment of the second aspect of the present invention, the chromatographic method is a liquid chromatographic method such as a HPLC, LC-MS or LC-MS/MS method; preferably the chromatographic method is a HPLC method.

The chromatographic method may be an isocratic method, preferably such that the relative concentration of the liquids A and B by volume is set between 99.5% A : 0.5% B and 0.5% A : 99.5% B, or between 90% A : 10% B and 10% A : 90% B, more preferably between 75% A : 25% B and 25% A : 75% B. More preferably still the relative concentration of the liquids A and B by volume is about 45% A : 55% B.
Alternately, the relative concentration of the liquids in the mobile phase may be varied to a predetermined gradient. Typically, the relative concentration of the liquids A and B by volume is varied to a gradient between 100 % A : 0 % B to 0 % A : 100 % B over a period of 10 to 180 minutes. Preferably the gradient is between 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 120 minutes, more preferably 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 60 minutes.

Alternatively, the chromatographic method of the second aspect of the present invention may comprise a gradient programming so that the relative concentration of the liquids A and B by volume starts at a first ratio, then is varied to a first gradient over a first period of time, to arrive at a second ratio, then optionally is maintained at said second ratio for a second period of time.

The first ratio may be 70-90 % A : 10-30 % B. Preferably the first ratio is 75-85 % A : 15-25 % B. Most preferably the first ratio is about 80 % A : 20 % B.

The first period of time may be from 0 to 120 minutes. Preferably the first period of time is from 15 to 60 minutes. Most preferably the first period of time is about 30 minutes.

The second ratio may be 0-20 % A : 80-100 % B. Preferably the second ratio is 5-15 % A : 85-95 % B. Most preferably the second ratio is about 10 % A : 90 % B.

The second period of time may be from 0 to 60 minutes. Preferably the second period of time is from 2 to 20 minutes. Most preferably the second period of time is about 5 minutes.

A particularly preferred method according to the second aspect of the present invention is when the first liquid A is 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol and the gradient is as follows:
<table>
<thead>
<tr>
<th>Time (min)</th>
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<tr>
<td>45</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Typically, the method of the second aspect of the present invention is carried out at a column temperature between approximately 15 to 40°C.

Preferably a mobile phase flow rate of between 0.01 and 10 ml/min is used, more preferably a mobile phase flow rate of between 0.1 and 4 ml/min is used, more preferably still a mobile phase flow rate of between 0.5 and 1.5 ml/min is used, most preferably a mobile phase flow rate of about 1 ml/min is used.

In one embodiment of the second aspect of the present invention, the stationary phase used is a gel, preferably a silica gel.

Preferably the stationary phase used in the second aspect of the present invention is reverse phase such as octadecylsilyl silica gel, octylsilyl silica gel, phenylalkyl silica gel, cyanopropyl silica gel, aminopropyl silica gel or an alkyl-diol silica gel. Particularly suitable stationary phases include octadecylsilyl silica gel or octylsilyl silica gel. A particularly preferred stationary phase comprises an Inertsil ODS 3V (250 mm x 4.6 mm), 5µm column.

Preferably the stationary phase has a particle size of between 0.1 and 10µm, or between 0.5 and 25µm, or between 1 and 10µm, or between 4.5 and 6µm. More preferably the stationary phase has a particle size of about 5µm.

Preferably the stationary phase has a pore size of between 10 and 100Å, or between 25 and 500Å, or between 50 and 200Å. More preferably the stationary phase has a pore size of between 75 and 125Å, or between 90 and 110Å. Most preferably the stationary phase has a pore size of about 100Å.
In one embodiment of the second aspect of the present invention, the chromatography is carried out in a column between 10mm and 5000mm in length, or in a column between 50mm and 1000mm in length, or between 100mm and 500mm in length. More preferably the chromatography is carried out in a column between 200mm and 280mm in length. Most preferably the chromatography is carried out in a column about 250mm in length.

The chromatography may be carried out in a column between 0.01mm and 100mm in internal diameter, or between 0.1mm and 50mm in internal diameter, or between 1mm and 10mm in internal diameter. More preferably the chromatography is carried out in a column about 4.6mm in internal diameter.

The eluent may be analysed by a detector such as a UV and/or visible spectrophotometer, a fluorescence spectrophotometer, a differential refractometer, an electrochemical detector, a mass spectrometer, a light scattering detector or a radioactivity detector.

In one embodiment of the second aspect of the present invention the chromatographic method detects and optionally quantifies in a single run one or more impurities selected from:

- 5-fluoro-2,3-dihydro-1H-indole-2-one; and
- N-[2-(cHethylamino)emyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

Preferably the chromatographic method detects and optionally quantifies in a single run both 5-fluoro-2,3-dihydro-1H-indole-2-one and N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

In a preferred embodiment the chromatographic method according to the second aspect of the present invention efficiently detects and quantifies in a single run all impurities including those selected from the following compounds:

- 5-fluoro-2,3-dihydro-1H-indole-2-one; and
- N-[2-(diethylamino)ethyl]-2,4-diethyl-5-formyl-1H-pyrrole-3-carboxamide.

In one embodiment of the second aspect of the present invention, 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-diethyl-5-formyl-1H-pyrrole-3-


carboxamide is used as internal or external reference marker, or as internal or external reference standard.

In another embodiment of the second aspect of the present invention, sunitinib, clozapine and/or ranitidine is used as internal or external reference marker, or as internal or external reference standard.

In one embodiment of the second aspect of the present invention, the sunitinib is in the form of a salt, solvate, hydrate or anhydrate. Preferably the method of the second aspect of the present invention is for analysing sunitinib malate. More preferably the method of the second aspect of the present invention is for analysing sunitinib (S)-malate. Optionally, the method of the second aspect of the present invention is for analysing anhydrous sunitinib, such as anhydrous sunitinib free base or anhydrous sunitinib (S)-malate.

In another embodiment of the second aspect of the present invention, the chromatographic method is used for the analysis of sunitinib or a salt thereof that is suitable for use in a pharmaceutical composition.

Preferably the chromatographic method is used for the analysis of sunitinib or a salt thereof that has not entered the human or animal body. Preferably the sunitinib or the salt thereof that is analysed is not in contact with a human or animal bodily fluid such as plasma. Preferably the sunitinib or the salt thereof that is analysed is not in contact with a human or animal bodily tissue such as liver, kidney, brain or white fat tissue. Preferably the sunitinib or the salt thereof that is analysed is not in solution.

In another embodiment of the second aspect of the present invention, the chromatographic method is used for the analysis of a pharmaceutical composition comprising sunitinib or a salt thereof.

In yet another embodiment of the second aspect of the present invention, the chromatographic method is used for the analysis of a substance comprising at least 5% sunitinib or a salt thereof by weight. Preferably the substance comprises at least 10%, at
least 25%, at least 50%, at least 75% or at least 90% sunitinib or a salt thereof by weight. Most preferably the substance comprises at least 95% sunitinib or a salt thereof by weight.

In yet another embodiment of the second aspect of the present invention, the chromatographic method is used for the analysis of a substance comprising sunitinib or a salt thereof as the only active pharmaceutical ingredient.

A third aspect of the present invention provides a chromatographic method for analysing sunitinib or a salt thereof, wherein the mobile phase comprises acetic acid.

Preferably the mobile phase comprises an aqueous solution of acetic acid. The acetic acid is typically present at a concentration of 0.001 to 0.2 % v/v, preferably at a concentration of 0.005 to 0.1 % v/v, more preferably at a concentration of 0.01 to 0.1 % v/v, and most preferably at a concentration of about 0.05 % v/v.

In one embodiment of the third aspect of the present invention, the aqueous solution further comprises an organic salt, an inorganic salt, an organic base or a mixture thereof. More preferably the aqueous solution further comprises an organic salt, an inorganic salt or a mixture thereof. Typically, the salt is a phosphate salt, an acetate salt, a trifluoroacetate salt, a formate salt, or a mixture thereof. The counter cation to the salt is preferably an ammonium cation.

Most preferably the salt is an acetate salt, such as ammonium acetate.

The salt in the aqueous solution can be present at a concentration of 0.001 to 0.2 M, preferably at a concentration of 0.005 to 1 M, more preferably at a concentration of 0.01 to 0.1 M, and most preferably at a concentration of about 0.05 M.

Preferably the pH of the aqueous solution is approximately 2 to 6.5. More preferably the pH of the aqueous solution is approximately 4 to 6. Most preferably the pH of the aqueous solution is about 5.5.
In one embodiment of the third aspect of the present invention, the mobile phase comprises two or more liquids, including a first liquid A and a second liquid B, wherein at least one of said liquids comprises the acetic acid. Preferably the first liquid A is an aqueous solution of the acetic acid. More preferably the first liquid A is an aqueous solution of a mixture of the acetic acid and a salt. Most preferably the first liquid A is an aqueous solution of a mixture of the acetic acid and an acetate salt such as ammonium acetate.

The second liquid B preferably comprises or is an organic solvent, preferably selected from an alkyl alcohol, such as methanol, ethanol, propanol or iso-propanol, or acetonitrile or a mixture thereof.

In one embodiment of the third aspect of the present invention, the second liquid B comprises or is a polar protic organic solvent such as formic acid, methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol, or a mixture thereof. Preferably the polar protic organic solvent is an alcohol such as a C₁-C₆ alcohol. More preferably the alcohol is an alkyl alcohol. More preferably still the alcohol is a C₃-C₄ alkyl alcohol such as methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol. Most preferably the second liquid B is methanol.

In another embodiment of the third aspect of the present invention, the second liquid B is substantially water miscible.

In one embodiment of the third aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% acetonitrile by volume. In one embodiment, the mobile phase contains no acetonitrile.

In another embodiment of the third aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% of any organic dipolar aprotic solvent by volume. In one embodiment, the mobile phase contains no organic dipolar aprotic solvent.
A preferred embodiment of the third aspect of the present invention is when the first liquid A is an aqueous solution of a buffer comprising an acetate salt mixed with acetic acid, and the second liquid B is methanol.

A particularly preferred embodiment of the third aspect of the present invention is when the first liquid A is a mixture of 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol.

In one embodiment of the third aspect of the present invention, the chromatographic method is a liquid chromatographic method such as a HPLC, LC-MS or LC-MS/MS method; preferably the chromatographic method is a HPLC method.

The chromatographic method may be an isocratic method, preferably such that the relative concentration of the liquids A and B by volume is set between 99.5 % A : 0.5 % B and 0.5 % A : 99.5 % B, or between 90 % A : 10 % B and 10 % A : 90 % B, more preferably between 75 % A : 25 % B and 25 % A : 75 % B. More preferably still the relative concentration of the liquids A and B by volume is about 45 % A : 55 % B.

Alternately, the relative concentration of the liquids in the mobile phase may be varied to a predetermined gradient. Typically, the relative concentration of the liquids A and B by volume is varied to a gradient between 100 % A : 0 % B to 0 % A : 100 % B over a period of 10 to 180 minutes. Preferably the gradient is between 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 120 minutes, more preferably 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 60 minutes.

Alternatively, the chromatographic method of the third aspect of the present invention may comprise a gradient programming so that the relative concentration of the liquids A and B by volume starts at a first ratio, then is varied to a first gradient over a first period of time, to arrive at a second ratio, then optionally is maintained at said second ratio for a second period of time.

The first ratio may be 70-90 % A : 10-30 % B. Preferably the first ratio is 75-85 % A : 15-25 % B. Most preferably the first ratio is about 80 % A : 20 % B.
The first period of time may be from 0 to 120 minutes. Preferably the first period of time is from 15 to 60 minutes. Most preferably the first period of time is about 30 minutes.

The second period of time may be from 0 to 60 minutes. Preferably the second period of time is from 2 to 20 minutes. Most preferably the second period of time is about 5 minutes.

A particularly preferred method according to the third aspect of the present invention is when the first liquid A is 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol and the gradient is as follows:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A (by volume)</th>
<th>% B (by volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
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<td>36</td>
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<td>20</td>
</tr>
<tr>
<td>45</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Typically, the method of the third aspect of the present invention is carried out at a column temperature between approximately 15 to 40°C.

Preferably a mobile phase flow rate of between 0.01 and 10 ml/min is used, more preferably a mobile phase flow rate of between 0.1 and 4 ml/min is used, more preferably still a mobile phase flow rate of between 0.5 and 1.5 ml/min is used, most preferably a mobile phase flow rate of about 1 ml/min is used.

In one embodiment of the third aspect of the present invention, the stationary phase used is a gel, preferably a silica gel.
Preferably the stationary phase used in the third aspect of the present invention is reverse phase such as octadecylsilyl silica gel, octylsilyl silica gel, phenylalkyl silica gel, cyanopropyl silica gel, aminopropyl silica gel or an alkyl-diol silica gel. Particularly suitable stationary phases include octadecylsilyl silica gel or octylsilyl silica gel. A particularly preferred stationary phase comprises an Inertsil ODS 3V (250 mm x 4.6 mm), 5µm column.

Preferably the stationary phase has a particle size of between 0.1 and 100µm, or between 0.5 and 25µm, or between 1 and 100µm, or between 4.5 and 60µm. More preferably the stationary phase has a particle size of about 5µm.

Preferably the stationary phase has a pore size of between 10 and 1000Å, or between 25 and 500Å, or between 50 and 200Å. More preferably the stationary phase has a pore size of between 75 and 125Å, or between 90 and HOA. Most preferably the stationary phase has a pore size of about 10Å.

In one embodiment of the third aspect of the present invention, the chromatography is carried out in a column between 10mm and 5000mm in length, or in a column between 50mm and 1000mm in length, or between 100mm and 500mm in length. More preferably the chromatography is carried out in a column between 200mm and 280mm in length. Most preferably the chromatography is carried out in a column about 250mm in length.

The chromatography may be carried out in a column between 0.01mm and 100mm in internal diameter, or between 0.1mm and 50mm in internal diameter, or between 1mm and 10mm in internal diameter. More preferably the chromatography is carried out in a column about 4.6mm in internal diameter.

The eluent may be analysed by a detector such as a UV and/ or visible spectrophotometer, a fluorescence spectrophotometer, a differential refractometer, an electrochemical detector, a mass spectrometer, a light scattering detector or a radioactivity detector.

In one embodiment of the third aspect of the present invention the chromatographic method detects and optionally quantifies in a single run one or more impurities selected from:
5-fluoro-2,3-dihydro-1H-indole-2-one; and
N-[2-(cHemylammo)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

Preferably the chromatographic method detects and optionally quantifies in a single run both 5-fluoro-2,3-dihydro-1H-indole-2-one and N-[2-(cHethylammo)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

In a preferred embodiment the chromatographic method according to the third aspect of the present invention efficiently detects and quantifies in a single run all impurities including those selected from the following compounds:
5-fluoro-2,3-dihydro-1H-indole-2-one; and
N-[2-(diemylaniino)ethyl]-2,4-dimemyl-5-formyl-1H-pyrrole-3-carboxamide.

In one embodiment of the third aspect of the present invention, 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide is used as internal or external reference marker, or as internal or external reference standard.

In another embodiment of the third aspect of the present invention, sunitinib, clozapine and/or ranitidine is used as internal or external reference marker, or as internal or external reference standard.

In one embodiment of the third aspect of the present invention, the sunitinib is in the form of a salt, solvate, hydrate or anhydrate. Preferably the method of the third aspect of the present invention is for analysing sunitinib malate. More preferably the method of the third aspect of the present invention is for analysing sunitinib (S)-malate. Optionally, the method of the third aspect of the present invention is for analysing anhydrous sunitinib, such as anhydrous sunitinib free base or anhydrous sunitinib (S)-malate.

In another embodiment of the third aspect of the present invention, the chromatographic method is used for the analysis of sunitinib or a salt thereof that is suitable for use in a pharmaceutical composition.
Preferably the chromatographic method is used for the analysis of sunitinib or a salt thereof that has not entered the human or animal body. Preferably the sunitinib or the salt thereof that is analysed is not in contact with a human or animal bodily fluid such as plasma. Preferably the sunitinib or the salt thereof that is analysed is not in contact with a human or animal bodily tissue such as liver, kidney, brain or white fat tissue. Preferably the sunitinib or the salt thereof that is analysed is not in solution.

In another embodiment of the third aspect of the present invention, the chromatographic method is used for the analysis of a pharmaceutical composition comprising sunitinib or a salt thereof.

In yet another embodiment of the third aspect of the present invention, the chromatographic method is used for the analysis of a substance comprising at least 5% sunitinib or a salt thereof by weight. Preferably the substance comprises at least 10%, at least 25%, at least 50%, at least 75% or at least 90% sunitinib or a salt thereof by weight. Most preferably the substance comprises at least 95% sunitinib or a salt thereof by weight.

In yet another embodiment of the third aspect of the present invention, the chromatographic method is used for the analysis of a substance comprising sunitinib or a salt thereof as the only active pharmaceutical ingredient.

A fourth aspect of the present invention provides a method for analysing a substance, comprising the detection and optional quantification of 5-fluoro-2,3-dihydro-1H-indole-2-one and/ or N-[2-<(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

Preferably the method of the fourth aspect of the present invention comprises the detection and optional quantification of both 5-fluoro-2,3-dihydro-1H-indole-2-one and N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

In one embodiment of the fourth aspect of the present invention, the method further comprises the detection and optional quantification of sunitinib or a salt thereof.
In another embodiment of the fourth aspect of the present invention, the substance is an active pharmaceutical ingredient. Preferably the substance is sunitinib, optionally in the form of a salt, solvate, hydrate or anhydride. More preferably the sunitinib is in the form of the malate salt. Most preferably the sunitinib is in the form of the (S)-malate salt.

Optionally the sunitinib is anhydrous, such as anhydrous sunitinib free base or anhydrous sunitinib (S)-malate. Preferably the sunitinib or the salt thereof analysed is for use in a pharmaceutical composition.

Preferably the substance that is analysed has not entered the human or animal body.

Preferably the substance that is analysed is not in contact with a human or animal bodily fluid such as plasma. Preferably the substance that is analysed is not in contact with a human or animal bodily tissue such as liver, kidney, brain or white fat tissue. Preferably the substance that is analysed is not in solution.

In another embodiment of the fourth aspect of the present invention, the method is a method of analysing a pharmaceutical composition comprising sunitinib or a salt thereof.

In yet another embodiment of the fourth aspect of the present invention, the substance comprises at least 5% sunitinib or a salt thereof by weight. Preferably the substance comprises at least 10%, at least 25%, at least 50%, at least 75% or at least 90% sunitinib or a salt thereof by weight. Most preferably the substance comprises at least 95% sunitinib or a salt thereof by weight.

In yet another embodiment of the fourth aspect of the present invention, the substance comprises sunitinib or a salt thereof as the only active pharmaceutical ingredient.

In a preferred embodiment of the fourth aspect of the present invention, the method is a chromatographic method, preferably wherein the mobile phase comprises two or more liquids, including a first liquid A and a second liquid B.

Preferably the first liquid A is aqueous based, such as water or an aqueous solution of a buffer. Preferably the buffer is selected from an acid, an organic salt, an inorganic salt, an
organic base or a mixture thereof. More preferably the buffer is selected from an acid, an organic salt, an inorganic salt or a mixture thereof.

Typically, the buffer is a phosphate salt, an acetate salt, a trifluoroacetate salt, a formate salt, acetic acid, trifluoroacetic acid, formic acid, a phosphoric acid such as orthophosphoric acid, or a mixture thereof. More typically, the buffer is a phosphate salt, an acetate salt, a formate salt, acetic acid, trifluoroacetic acid, or a mixture thereof.

Where the buffer is a salt, preferably the counter cation is an ammonium cation.

Most preferably the buffer is a mixture of an acetate salt, such as ammonium acetate, and an acid, more preferably an organic acid, such as acetic acid.

The salt in the buffer can be present at a concentration of 0.001 to 0.2 M, preferably at a concentration of 0.005 to 0.1 M, more preferably at a concentration of 0.01 to 0.1 M, and most preferably at a concentration of about 0.05 M; and/or the acid can be present at a concentration of 0.001 to 0.2 % v/v, preferably at a concentration of 0.005 to 0.1 % v/v, more preferably at a concentration of 0.01 to 0.1 % v/v, and most preferably at a concentration of about 0.05 % v/v.

Preferably the buffer is a mixture of ammonium acetate present at a concentration of 0.01 to 0.1 M and acetic acid at a concentration of 0.01 to 0.1 % v/v. Most preferably the buffer is a mixture of ammonium acetate present at a concentration of about 0.05 M and acetic acid at a concentration of about 0.05 % v/v.

Preferably the pH of the buffer solution is approximately 2 to 6.5. More preferably the pH of the buffer solution is approximately 4 to 6. Most preferably the pH of the buffer solution is about 5.5.

The second liquid B preferably comprises or is an organic solvent, preferably selected from an alkyl alcohol, such as methanol, ethanol, propanol or iso-propanol, or acetonitrile or a mixture thereof.
In one embodiment of the fourth aspect of the present invention, the second liquid B comprises or is a polar protic organic solvent such as acetic acid, methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol, or a mixture thereof. Preferably the polar protic organic solvent is an alcohol such as a C₁-C₆ alcohol. More preferably the alcohol is an alkyl alcohol. More preferably still the alcohol is a C₁-C₄ alkyl alcohol such as methanol, ethanol, n-propanol, n-butanol, iso-propanol, iso-butanol, sec-butanol or tert-butanol. Most preferably the second liquid B is methanol.

In another embodiment of the fourth aspect of the present invention, the second liquid B is substantially water miscible.

In one embodiment of the fourth aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% acetonitrile by volume. In one embodiment, the mobile phase contains no acetonitrile.

In another embodiment of the fourth aspect of the present invention, the mobile phase contains less than 10%, less than 5% or less than 1% of any organic dipolar aprotic solvent by volume. In one embodiment, the mobile phase contains no organic dipolar aprotic solvent.

A preferred embodiment of the fourth aspect of the present invention is when the first liquid A is an aqueous solution of a buffer comprising an acetate salt mixed with acetic acid, and the second liquid B is methanol.

A particularly preferred embodiment of the fourth aspect of the present invention is when the first liquid A is a mixture of 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol.

In one embodiment of the fourth aspect of the present invention, the chromatographic method is a liquid chromatographic method such as a HPLC, LC-MS or LC-MS/MS method; preferably the chromatographic method is a HPLC method.
Preferably in any chromatographic method of the fourth aspect of the present invention, said method detects and optionally quantifies in a single run 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide. More preferably said method also detects and optionally quantifies in the same run sunitinib or a salt thereof.

Most preferably said method detects and optionally quantifies in a single run all three of: sunitinib or a salt thereof; 5-fluoro-2,3-dihydro-1H-indole-2-one; and N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

In one embodiment of the fourth aspect of the present invention, 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide is used as internal or external reference marker, or as internal or external reference standard.

In another embodiment of the fourth aspect of the present invention, sunitinib, clozapine and/or ranitidine is used as internal or external reference marker, or as internal or external reference standard.

The chromatographic method may be an isocratic method, preferably such that the relative concentration of the liquids A and B by volume is set between 99.5 % A : 0.5 % B and 0.5 % A : 99.5 % B, or between 90 % A : 10 % B and 10 % A : 90 % B, more preferably between 75 % A : 25 % B and 25 % A : 75 % B. More preferably still the relative concentration of the liquids A and B by volume is about 45 % A : 55 % B.

Alternately, the relative concentration of the liquids in the mobile phase may be varied to a predetermined gradient. Typically, the relative concentration of the liquids A and B by volume is varied to a gradient between 100 % A : 0 % B to 0 % A : 100 % B over a period of 10 to 180 minutes. Preferably the gradient is between 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 120 minutes, more preferably 100 % A : 0 % B to 0 % A : 100 % B over a period of 25 to 60 minutes.
Alternatively, the chromatographic method of the fourth aspect of the present invention may comprise a gradient programming so that the relative concentration of the liquids A and B by volume starts at a first ratio, then is varied to a first gradient over a first period of time, to arrive at a second ratio, then optionally is maintained at said second ratio for a second period of time.

The first ratio may be 70-90 % A : 10-30 % B. Preferably the first ratio is 75-85 % A : 15-25 % B. Most preferably the first ratio is about 80 % A : 20 % B.

The first period of time may be from 0 to 120 minutes. Preferably the first period of time is from 15 to 60 minutes. Most preferably the first period of time is about 30 minutes.

The second ratio may be 0-20 % A : 80-100 % B. Preferably the second ratio is 5-15 % A : 85-95 % B. Most preferably the second ratio is about 10 % A : 90 % B.

The second period of time may be from 0 to 60 minutes. Preferably the second period of time is from 2 to 20 minutes. Most preferably the second period of time is about 5 minutes.

A particularly preferred method according to the fourth aspect of the present invention is when the first liquid A is 0.05 M ammonium acetate containing 0.05 % v/v acetic acid and the second liquid B is methanol and the gradient is as follows:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A (by volume)</th>
<th>% B (by volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
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<tr>
<td>35</td>
<td>10</td>
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<td>80</td>
<td>20</td>
</tr>
<tr>
<td>45</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Typically, the method of the fourth aspect of the present invention is carried out at a column temperature between approximately 15 to 40°C.
Preferably a mobile phase flow rate of between 0.01 and 10 ml/min is used, more preferably a mobile phase flow rate of between 0.1 and 4 ml/min is used, more preferably still a mobile phase flow rate of between 0.5 and 1.5 ml/min is used, most preferably a mobile phase flow rate of about 1 ml/min is used.

In one embodiment of the fourth aspect of the present invention, the stationary phase used is a gel, preferably a silica gel.

Preferably the stationary phase used in the fourth aspect of the present invention is reverse phase such as octadecylsilyl silica gel, octylsilyl silica gel, phenylalkyl silica gel, cyanopropyl silica gel, aminopropyl silica gel or an alkyl-diol silica gel. Particularly suitable stationary phases include octadecylsilyl silica gel or octylsilyl silica gel. A particularly preferred stationary phase comprises an Inertsil ODS 3V (250 mm x 4.6 mm), 5µm column.

Preferably the stationary phase has a particle size of between 0.1 and 10µm, or between 0.5 and 25µm, or between 1 and 10µm, or between 4.5 and 6µm. More preferably the stationary phase has a particle size of about 5µm.

Preferably the stationary phase has a pore size of between 10 and 1000Å, or between 25 and 500Å, or between 50 and 200Å. More preferably the stationary phase has a pore size of between 75 and 125Å, or between 90 and 100Å. Most preferably the stationary phase has a pore size of about 100Å.

In one embodiment of the fourth aspect of the present invention, the chromatography is carried out in a column between 10mm and 5000mm in length, or in a column between 50mm and 1000mm in length, or between 100mm and 500mm in length. More preferably the chromatography is carried out in a column between 200mm and 280mm in length. Most preferably the chromatography is carried out in a column about 250mm in length.

The chromatography may be carried out in a column between 0.01mm and 100mm in internal diameter, or between 0.1mm and 50mm in internal diameter, or between 1mm and 10mm in internal diameter. More preferably the chromatography is carried out in a column about 4.6mm in internal diameter.
The eluent may be analysed by a detector such as a UV and/or visible spectrophotometer, a fluorescence spectrophotometer, a differential refractometer, an electrochemical detector, a mass spectrometer, a light scattering detector or a radioactivity detector.

A fifth aspect of the present invention provides a process for preparing a batch of a substance, said process comprising the steps of:

(i) providing a source quantity of the substance;
(ii) removing a sample from said source quantity and subjecting said sample to a method according to any of the first to fourth aspects of the present invention; and
(iii) retaining some or all of the remainder of said source quantity to give the batch of the substance.

In one embodiment of the fifth aspect of the present invention, the substance comprises or is an active pharmaceutical ingredient. Preferably the substance comprises or is sunitinib, optionally in the form of a salt, solvate, hydrate or anhydrate. More preferably the sunitinib is in the form of the malate salt. Most preferably the sunitinib is in the form of the (S)-malate salt. Optionally the sunitinib is anhydrous, such as anhydrous sunitinib free base or anhydrous sunitinib (S)-malate. Preferably the substance is for use in a pharmaceutical composition.

In another embodiment of the fifth aspect of the present invention, the substance comprises or is a pharmaceutical composition. Preferably the pharmaceutical composition comprises sunitinib, optionally in the form of a salt, solvate, hydrate or anhydrate. More preferably the sunitinib is in the form of the malate salt. Most preferably the sunitinib is in the form of the (S)-malate salt. Optionally the sunitinib is anhydrous, such as anhydrous sunitinib free base or anhydrous sunitinib (S)-malate. Preferably the pharmaceutical composition comprises one or more pharmaceutically acceptable excipients.

Preferably the substance of the fifth aspect of the present invention has not entered the human or animal body. Preferably the substance is not in contact with a human or animal bodily fluid such as plasma. Preferably the substance is not in contact with a human or
animal bodily tissue such as liver, kidney, brain or white fat tissue. Preferably the substance is not in solution.

In another embodiment of the fifth aspect of the present invention, the substance comprises at least 5% sunitinib or a salt thereof by weight. Preferably the substance comprises at least 10%, at least 25%, at least 50%, at least 75% or at least 90% sunitinib or a salt thereof by weight. Most preferably the substance comprises at least 95% sunitinib or a salt thereof by weight.

In yet another embodiment of the fifth aspect of the present invention, the substance comprises sunitinib or a salt thereof as the only active pharmaceutical ingredient.

A sixth aspect of the present invention provides a batch of sunitinib or a salt thereof which has been prepared by a process according to the fifth aspect of the present invention. Preferably the sunitinib is substantially free of 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide.

Sunitinib or a salt thereof is "substantially free" of a compound, if it comprises less than about 5% of that compound, preferably less than about 3%, preferably less than about 2%, preferably less than about 1%, preferably less than about 0.5%, preferably less than about 0.1%, preferably less than about 0.05%, preferably as measured by HPLC.

A seventh aspect of the present invention provides a process for preparing a pharmaceutical composition, said process comprising the step of combining one or more pharmaceutically acceptable excipients with part or all of a batch of sunitinib or a salt thereof which has been prepared by a process according to the fifth aspect of the present invention.

An eighth aspect of the present invention provides a pharmaceutical composition prepared by a process according to the seventh aspect of the present invention.

A ninth aspect of the present invention provides a batch of one or more pharmaceutical compositions which have been prepared by a process according to the fifth aspect of the
present invention, wherein the pharmaceutical composition(s) comprise sunitinib or a salt thereof. Preferably the pharmaceutical composition(s) also comprise one or more pharmaceutically acceptable excipients.

For the avoidance of doubt, insofar as is practicable any embodiment of a given aspect of the present invention may occur in combination with any other embodiment of the same aspect of the present invention. In addition, insofar as is practicable it is to be understood that any preferred or optional embodiment of any aspect of the present invention should also be considered as a preferred or optional embodiment of any other aspect of the present invention.

Detailed description of the present invention

The present invention can be used to analyse sunitinib and/or its salts, in particular sunitinib malate, as an API or when prepared as a pharmaceutical composition.

The pharmaceutical compositions that can be analysed by the present invention include solid and liquid compositions and optionally comprise one or more pharmaceutically acceptable carriers or excipients. Solid form compositions include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. Liquid compositions include solutions or suspensions which can be administered by oral, injectable, inhalation or infusion routes.

The term "impurities" or "related substances" as used herein throughout the specification can mean either impurities formed in the manufacture of the API or the pharmaceutical composition and/or formed by degradation of the API or in the pharmaceutical composition on storage.

As discussed above, the HPLC methods reported in the prior art are not suitable for analysing sunitinib, particularly with respect to the related substances formed in the synthesis of sunitinib and/or its salts prepared by the process disclosed in patent US 6573293.
However, the present invention solves this problem and efficiently detects and quantifies, in a single run, all impurities and intermediates formed in this particular synthetic process. The present invention is advantageous as the gradient method allows the elution of all polar to non-polar impurities. Identification of all impurities in a single run is particularly advantageous and cost saving in a commercial environment.

The present invention is also advantageous as the method is selective, linear and precise for the analysis of related substances in sunitinib and/or its salts. In addition, the present invention is highly sensitive and allows detection and quantification of related substances in sunitinib and/or its salts at levels much lower than acceptance limits specified by health authorities.

In addition, the method of the present invention can be used to easily detect and quantify all degradation impurities formed on storage of samples of sunitinib. This was established by carrying out forced degradation studies as per ICH Q1A (R2) Guidelines and validated as per ICH Q2C (R1) Guidelines covering the parameters Specificity, Linearity and Range, Precision (Reproducibility), Limit of Detection (LOD), Limit of Quantitation (LOQ) and System Suitability.

The buffer optionally used in the first liquid A can be an inorganic salt such as sodium, potassium, calcium, magnesium, lithium or aluminium salts of phosphate, acetate or formate and mixtures thereof. Alternatively the buffer can be an organic salt such as the ammonium salt of acetate or formate and mixtures thereof. Alternatively the buffer can be a mineral acid or a carboxylic acid, such as acetic acid or trifluoroacetic acid. Most preferably the first liquid A is a mixture of 0.05 M ammonium acetate and 0.05 % v/v acetic acid.

The second liquid B is an organic solvent such as an alcohol, preferably a C<sub>1</sub> to C<sub>6</sub> alkyl alcohol like methanol, ethanol, propanol, butanol or iso-propanol or mixtures thereof. Alternatively, the organic solvent(s) may be tetrahydrofuran, ethyl acetate or acetonitrile or any suitable organic solvent(s). Most preferably the organic solvent is methanol.
Preferably the stationary phase used in the method of the present invention is selected from octadecylsilyl silica gel (RP-18) or octylsilyl silica gel (RP-8).

An internal standard reference compound may be used in the method of the present invention if required. Alternatively the concentration of the components analysed may be determined by comparison with one or more external reference compounds.

The inventors have tested the methods of the present invention extensively to show that they are reproducible, precise and linear with respect to concentration.

While the present invention has been described in terms of its specific embodiments, certain modifications and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the present invention.

The present invention is illustrated but in no way limited by the following example.

**Example**

**Experimental conditions:**

- Column: Inertsil ODS 3V (250 mm x 4.6 mm), 5µ; 10Å pore size;
- Flow rate: 1 ml/min;
- Detection: 235 nm;
- Sample concentration: 1000 ppm;
- Diluent: [0.05 M ammonium acetate + 0.05 % acetic acid] - methanol (65:35 v/v);

The sample of sunitinib (S)-malate (anhydrous) is initially dissolved in a small volume of the diluent; the sample solution is then injected into the column which is run using the mobile phase outlined below;

First Liquid A: a mixture of 0.05 M ammonium acetate and 0.05 % v/v acetic acid;
Second Liquid B: methanol;

Mobile phase: First Liquid A - Second Liquid B gradient; the gradient program is described below, with the program between 35 and 45 minutes being used to equilibrate and prepare the column for the next run:
Retention times (RT), Relative retention times (RRT), Limit of Detection (LOD) and Limit of Quantitation (LOQ) obtained for the impurities and sunitinib are given in Table 1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Approximate RT (min)</th>
<th>Approximate RRT</th>
<th>LOD (%)</th>
<th>LOQ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-[2-(Diethylamino)ethyl]-2,4-dimethyl-5-formyl-1H-pyrrole-3-carboxamide</td>
<td>6.3</td>
<td>0.28</td>
<td>0.005</td>
<td>0.015</td>
</tr>
<tr>
<td>5-Fluoro-2,3-dihydro-1H-indole-2-one</td>
<td>16.1</td>
<td>0.71</td>
<td>0.005</td>
<td>0.015</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>22.7</td>
<td>1.00</td>
<td>0.015</td>
<td>0.045</td>
</tr>
</tbody>
</table>

* % LOD and LOQ values are with respect to sample concentration of 1000 ppm.

It will be understood that the present invention has been described above by way of example only. The examples are not intended to limit the scope of the invention. Various modifications and embodiments can be made without departing from the scope and spirit of the invention, which is defined by the following claims only.
Claims

1. A HPLC method for analysing sunitinib or a salt thereof, wherein the mobile phase comprises two or more liquids and the relative concentration of the liquids is varied to a predetermined gradient.

2. A HPLC method according to claim 1, wherein the mobile phase comprises a first liquid A which is aqueous based.

3. A HPLC method according to claim 2, wherein the first liquid A comprises water or an aqueous solution of a buffer.

4. A HPLC method according to claim 3, wherein the buffer is selected from an acid, an organic salt, an inorganic salt or a mixture thereof.

5. A HPLC method according to claim 4, wherein the buffer is selected from a phosphate salt, an acetate salt, a trifluoroacetate salt, a formate salt, trifluoroacetic acid, acetic acid, formic acid, a phosphoric acid such as orthophosphoric acid, or a mixture thereof.

6. A HPLC method according to claim 4 or 5, wherein the buffer is a mixture of an acetate salt and an acid.

7. A HPLC method according to claim 6, wherein the buffer is a mixture of ammonium acetate and acetic acid.

8. A HPLC method according to claim 6 or 7, wherein:
   (i) the acetate salt is present at a concentration of 0.001 to 0.2 M and/or the acid at a concentration of 0.001 to 0.2 % v/v; and/or
   (ii) the acetate salt is present at a concentration of 0.005 to 0.1 M and/or the acid at a concentration of 0.005 to 0.1 % v/v; and/or
   (iii) the acetate salt is present at a concentration of 0.01 to 0.1 M and/or the acid at a concentration of 0.01 to 0.1 % v/v; and/or
(iv) the acetate salt is present at a concentration of about 0.05 M and/or the acid at a concentration of about 0.05 % v/v.

9. A HPLC method according to claim 8, wherein:

(i) the acetate salt is ammonium acetate present at a concentration of 0.001 to 0.2 M and/or the acid is acetic acid at a concentration of 0.001 to 0.2 % v/v; and/or

(ii) the acetate salt is ammonium acetate present at a concentration of approximately 0.05 M and/or the acid is acetic acid at a concentration of approximately 0.05 % v/v.

10. A HPLC method according to any preceding claim, wherein the mobile phase comprises a second liquid B which is or comprises an organic solvent.

11. A HPLC method according to claim 10, wherein the second liquid B is an alcohol, preferably an alkyl alcohol, preferably selected from methanol, ethanol, propanol or isopropanol, or acetonitrile, or a mixture thereof.

12. A HPLC method according to claim 11, wherein the second liquid B is methanol.

13. A HPLC method according to any one of claims 10 to 12, wherein the first liquid A is a mixture of ammonium acetate and acetic acid and the second liquid B is methanol.

14. A HPLC method according to any one of claims 10 to 13, which comprises a gradient programming so that the relative concentration of the liquids A and B is varied to a gradient between 100 % A : 0 % B to 0 % A : 100 % B run over:

(i) 10 to 180 minutes; and/or

(ii) 25 to 120 minutes; and/or

(iii) 25 to 60 minutes.

15. A HPLC method according to any preceding claim, wherein the stationary phase used:

(i) is reverse phase; and/or

(ii) is octadecylsilyl silica gel or octylsilyl silica gel; and/or
(iii) comprises an Inertsil ODS 3V (250 mm x 4.6 mm), 5µ column.

16. A HPLC method according to any one of claims 10 to 15, wherein the first liquid A is a mixture of 0.05 M aqueous ammonium acetate and 0.05 % v/v acetic acid and the second liquid B is methanol.

17. A HPLC method according to claim 16, wherein the gradient is as follows:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A (by volume)</th>
<th>% B (by volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>35</td>
<td>10</td>
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<tr>
<td>36</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>45</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

18. A HPLC method according to any one of claims 3 to 17, wherein the pH of the buffer solution is approximately 2 to 6.5.

19. A HPLC method according to any preceding claim, wherein the chromatography is carried out at a temperature between approximately 15 to 40°C.

20. A HPLC method according to any preceding claim, wherein the sunitinib is in the form of any salt, solvate, hydrate or anhydrous.

21. A HPLC method according to claim 20, wherein the sunitinib is anhydrous.

22. A HPLC method according to any preceding claim, which detects and optionally quantifies in a single run one or more impurities selected from: 5-fluoro-2,3-dihydro-IH-indole-2-one; and N-[2-(diethylamino)ethyl]-2,4-dimetiyyl-5-formyl-IH-pyrrole-3-carboxamide.

23. A HPLC method according to any preceding claim, which efficiently detects and quantifies in a single run all impurities including those selected from the following compounds:
5-fluoro-2,3-dimethyl-1H-indole-2-one;
N-[2-(dimethylamino)ethyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide;
and any other unknown impurities.

24. A HPLC method according to any preceding claim, which is used to analyse sunitinib or a salt thereof as an API or when prepared as a pharmaceutical composition.

25. A chromatographic method for analysing sunitinib or a salt thereof, wherein the mobile phase comprises an alcohol.

26. A chromatographic method for analysing sunitinib or a salt thereof, wherein the mobile phase comprises acetic acid.

27. A method for analysing a substance, comprising the detection and optional quantification of 5-fluoro-2,3-dihydro-1H-indole-2-one and/or N-[2-(dimethylamino)ethyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide.

28. A process for preparing a batch of a substance, said process comprising the steps of:
   (i) providing a source quantity of the substance;
   (ii) removing a sample from said source quantity and subjecting said sample to a method according to any preceding claim; and
   (iii) retaining some or all of the remainder of said source quantity to give the batch of the substance.

29. A batch of sunitinib or a salt thereof, or a batch of one or more pharmaceutical compositions comprising sunitinib or a salt thereof, which has been prepared by a process according to claim 28.

30. A process for preparing a pharmaceutical composition, said process comprising the step of combining one or more pharmaceutically acceptable excipients with part or all of a batch of sunitinib or a salt thereof which has been prepared by a process according to claim 28.
31. A pharmaceutical composition which has been prepared by a process according to claim 30.
A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N30/34 C07D403/06 A61K31/33 C07D207/34 A61K31/404

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>5-15, 18</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"Q" document member of the same patent family

Date of the actual completion of the international search 29 April 2011
Date of mailing of the international search report 13/07/2011

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer
Marembert, Vincent

Form PCT/ISA/210 (second sheet) (April 2005)
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<td>w0 2009/128083 AI (NATCO PHARMA LTD [IN] ; KONDURI SRINIVASA KRISHNA MURT [IN] ; ADIBHATLA) 22 October 2009 (2009-10-22) claim 22</td>
<td>27</td>
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INTERNATIONAL SEARCH REPORT

Box No. Il Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1-27

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☒ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/21 0 (continuation of first sheet (2)) (April 2005)
FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-27
   A chromatographic method for analyzing sunitinib

2. claims: 28-31
   A process for preparing a batch of a substance
<table>
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<th>Publication date</th>
<th>Patent family member(s)</th>
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<tr>
<td>US 2009318525 A1</td>
<td>24-12-2009</td>
<td>AR 072117 A1</td>
<td>04-08-2010</td>
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<td>WO 2009150523 A1</td>
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