3,5,3'-TRIODOTHYRONINE SULFATE AS THYROMIMETIC AGENT AND PHARMACEUTICAL FORMULATIONS THEREOF

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The invention regards the use of triiodothyronine sulfate, commonly named T₃-S, as a medicament having thyromimetic activity for the treatment of pathologies due to organic deficiency of triiodothyronine (T₃), as such or in association with thyroxine (T₄), and pharmaceutical formulations for oral administration thereof.

Panel a)

Panel b)
Figure 1 Panel a)

\[ y = -2E-05x^2 + 0.0031x^2 - 0.1985x + 6.3253 \]

\[ R^2 = 0.9837 \]

Figure 1 Panel b)

\[ y = 11.882x^2 - 65.034x^2 + 114.33x - 61.988 \]

\[ R^2 = 0.9713 \]
Figure 2
Figure 3

Figure 4
3,5,3'-TRIOIODOTHYRONINE SULFATE AS THYROMETIC AGENT AND PHARMACEUTICAL FORMULATIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part (CIP) of U.S. Ser. No. 10/532,447 filed Apr. 22, 2005, which is a U.S. national phase application of PCT/EP2003/12584 filed Nov. 11, 2003, which in turn claims priority from M12002A002594 IT filed Nov. 13, 2002, the contents of each of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention regards the use of 3,5,3'-triiodothyronine sulfate, usually named triiodothyronine sulfate or T₃ sulfate (T₃S), as an active principle, alone or in combination with thyroxine, in the treatment of pathologies due to organic deficiency of 3,5,3'-triiodothyronine. Accordingly, the same is usable for the preparation of thyrometic pharmaceutical compositions.

BACKGROUND OF THE INVENTION

[0003] A number of iodothyronines are present in blood, which are directly produced by thyroid gland or are the result of peripheral metabolism of other iodothyronines. Among them, 3,5,3'-triiodothyronine (acronym T₃) is deemed to be the biological active form of thyroid hormone (T₄), because it has shown high affinity for the specific receptors of the same and is normally present in serum at a concentration sufficient for the activation of said receptors.

[0004] The main secretion product of thyroid gland in the healthy adult is thyroxine, commonly designated with the acronym T₄. It is peripherally converted to its biologically active form, T₃ (Ref. 1), through enzymatic removal of an iodine atom from the external aromatic ring of the molecule by both type I and type II 5'-iodothyronine monodeiodinases (type I MD and type II MD, respectively). This metabolic pathway is the main mechanism of endogenous production of T₃; thus, T₄ can properly be considered a pro-hormone. On the other hand, a minor part of T₃ is also directly secreted by thyroid gland. On average, the amount of T₃ produced in an adult being of 70 Kg weight every day amounts to 100 μg, while the total production of T₃ amounts to around 25 μg. 4.8 μg of T₃ out of said 25 μg are directly secreted by thyroid and the remaining ones derive from the peripheral conversion of T₄.

[0005] T₃ undergoes two different metabolic pathways. The main metabolic pathway consists in the partial deiodination of the outer aromatic ring by type III 5'-iodothyronine monodeiodinase (type III MD) to give 3,3'-diodothyronine, which is biologically non-active and is further metabolized through deiodination or sulfoconjugation. The other metabolic pathway regards around 20% of the total amount of T₃ produced by the body and brings on sulfoconjugation of T₃ to give T₃S, which is not able to bond to the thyroid hormone receptor (Ref. 2), thus resulting in biologically non-active (Ref. 3).

[0006] Contrary to what happens with T₄, T₃S is not deiodinated by type III MD. Rather, it is an excellent substrate for type I MD (Ref. 4), which converts it very quickly into 3,3'-diiodothyronine sulfate. Thus, it has been widespread common knowledge that, in the healthy adult being, sulfoconjugation of T₃ to give T₃S represents a way for speeding up the catabolism of T₃, so facilitating its biliary and urinary excretion. Actually, it was found that serum levels of T₃S, physiologically low in the healthy adult, are higher when type I MD activity is reduced.

[0007] Yet, it was found that, in some body districts and organs, sulfates exist which, under particular physiological conditions and situations, are able to convert again T₃S into its active form T₃ (Ref’s. 7-9).

[0008] Such enzymes have been described in the intestinal microflora as well as in body tissues like liver, kidneys and nervous central system (Ref. 10).

[0009] Recently, it has been found that endogenous T₃S levels in serum are quite high during intruterine life and as such are kept by the body, i.e. higher than the ones normally found in the adult being, at least until the forth month of postnatal life (Ref. 11). Considering the essential role played by thyroid hormones during growth, in particular as far as nervous central system functions are concerned, hypotheses have been made about the possibility that, in this tissue, T₃S may also possibly be used by the body as an occasional source of T₃, if and when needed, during the first period of life. Studies performed on autopic specimens of human nervous central tissue post-mortem showed that the amount of T₃ in the same limits predicted by type III MD (Ref. 12). While this enzyme does not attack T₃S, it has been assayed that T₃S may exceptionally represent an alternative endogenous source of T₃, both in those tissues which contain sulfates able to recover T₃S into its active form, just in case a particular need of the hormone arises in said tissues (Ref’s. 8, 13).

[0010] Further studies have been performed, aimed at ascertaining the effective role played by T₃S during production and metabolism of thyroid hormones. Said studies have recently demonstrated that when administered by intraperitoneal (i.p.) administration in single or 3 to 10 daily doses a thyrometric effect is observed in hypothyroid rats (Ref. 10). In euthyroid rats (Ref. 14) T3S, administered i.p., shows a thyrometric effect on several parameters such as body weight and TSH serum levels.

[0011] In both references T₃S has shown a potency of around one fifth that of T₃. Moreover both treatments with T₃S and with T₃ produced a significant reduction of serum levels of thyreotropic hormone (TSH) in euthyroid rats, thus showing to possess similar capability in inhibiting its secretion. On the contrary, in the case of hypothyroid rats, T₃S showed a poor capability of inhibiting TSH secretion when compared to T₃. It is well known that TSH is a highly responsive indicator to the functional status of thyroid gland and detects the smallest alterations of its hormonal secretion. Actually, its levels are higher under conditions of reduced thyroid functionality, even in those conditions that are defined as sub-clinical, while they are reduced when an excess of thyroid hormones are present. As a consequence, T₃S activity seems non-comparable to T₃ as far as its capability of inhibition on formation of TSH is involved.

[0012] Therefore, particularly in view of the latest studies the biological role of T₃S is still controversial.

[0013] In fact, its main, well-grounded and universally accepted, feature is its non-biological activity, i.e. it is a biologically inert metabolite of T₃ (Ref’s. 2 and 3), and the sulfation pathway is regarded as a metabolic activator of T₃ catabolism (Ref. 5).
On the other hand, only in particular tissues and under exceptional critical conditions due to shortage of thyroid hormone in those tissues, it has been shown its potential as an endogenous local source of \( T_3 \).

As a result, today the skilled technician is still facing a complex, somewhat conflicting, situation, which highlights only some of the biological characteristics of the product and needs more exhaustive in-depth studies.

To the best of our knowledge, however, none of the several documents forming the state-of-the-art discloses, shows or suggests the possibility of using this metabolite of \( T_3 \) in therapy. No close prior-art document, either of experimental nature or substantially speculative, either taken alone or in combination with other related documents, suggests the use, or even the potential use of \( T_3 \) as a medicament, taken as such or preferably in combination with other thyroid hormones or pro-hormones, like, for example, \( T_4 \). The fact that, only in some specific tissues of the body and under particular, peculiar circumstances, part of \( T_3 \) can be reconverted into \( T_4 \) does not mean, nor implies, nor suggests that it is possible to generalize this feature to the whole organism through exogenous administration of the product. In particular, there is no suggestion that oral administration of the product, even in protected form according to known methods of the pharmaceutical technique, may render it bioavailable also because it is well known that in those districts where suitable sulfatases are not present the same is rapidly metabolized and excreted through the bile and urines.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1.** Panel a) \( T_3 \) calibration curve by competitive ELISA; Panel b) DELFIA calibration curve. \( T_3 \) was assayed at 33.6, 56, 93.3, 155.5, 259, 432, 720, 1200, 2000 pg/ml.

**FIG. 2.** Schematic of DTPA-\( T_3 \) monoamide synthesis.

**FIG. 3.** Mean serum concentrations of \( T_3 \) (ng/dl) levels in the four doses groups (native values).

**FIG. 4.** Mean serum concentration curves of \( T_3 \) and Total \( T_3 \) after administration at a dose of 160 \( \mu \)g.

**SUMMARY OF THE INVENTION**

It has now unexpectedly been found, and this is one of the aspects of the present invention, that \( T_3 \) (triiodothyronine or 3,5-diiodo-O-[3-iodo-4-(sulphoxy)phenyl]-L-thyronine), as the only active principle in a suitable composition or in association with other thyroid hormones or pro-hormones, preferably \( T_3 \) (L-tyrosine, or 3,5,3',5'-tetraiodothyronine) and properly formulated according to the desired application for oral administration is particularly useful as a medicament to be used in all those pathologies caused by insufficient production by the body of the needed quantities of active thyroid hormones, in particular \( T_3 \).

Another aspect of the present invention is a non-radioactive immunoassay for \( T_3 \) quantitation, preferably based on chemiluminescence and the reagents developed therein.

A further object of the present invention is a method for the therapeutic treatment of a hypothyroid condition, which comprises the oral administration of \( T_3 \) or the combination \( T_3 \) and \( T_4 \) as a thyroid hormone replacement therapy to a subject in need thereof.

Accompanied by a preferred embodiment oral administration is accomplished by solid compositions, preferably in the tablet form, comprising \( T_3 \) alone or in combination with a second active principle, tyrosine (\( T_2 \)), in a dose comprised for \( T_3 \) of from 1 to 1000 \( \mu \)g, preferably 2.5-500 \( \mu \)g and \( T_2 \) of from 1 to 800 \( \mu \)g.

Preferred active principle quantities in the formulation comprising two active principles, are the following: \( T_3 \) 5-250 \( \mu \)g and \( T_3 \) 5-400 \( \mu \)g, \( T_3 \) 10-100 \( \mu \)g and \( T_2 \) 10-200 \( \mu \)g.

In any case, the preferred ratio between active principles (\( T_3 \) : \( T_2 \)) is comprised to from 10:1 to 0:1:1, with a more preferred range comprised from 5:1 to 1:1. Even more preferred is the range comprised from 3:1 to 2:1 (\( T_3 \) : \( T_2 \)).

Said compositions include diluents, glidants or lubricants and disintegrants and in a preferred embodiment consist essentially of: calcium carbonate, glycerol dibenenate, croscarmellose sodium salt, hydride colloidal silica, magnesium stearate and microcrystalline cellulose, in an even more preferred embodiment these components are present in the amounts described below for a 80-150 mg tablet:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium carbonate</td>
<td>20-40 mg, preferably 25-35 mg, more preferably 30 mg</td>
</tr>
<tr>
<td>Glycerol dibenenate</td>
<td>2-15 mg, preferably 4-8 mg, more preferably 5 mg</td>
</tr>
<tr>
<td>Croscarmellose sodium salt</td>
<td>1-10 mg, preferably 2-6 mg, more preferably 3.5 mg</td>
</tr>
<tr>
<td>Hydride colloidal silica</td>
<td>0.1-5 mg, preferably 0.5-4, more preferably 2 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.01-2 mg, preferably 0.1-1 mg, more preferably 0.5 mg</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Up to 110 mg</td>
</tr>
</tbody>
</table>

This composition is endowed with optimal dissolution rates and stability of the active principle(s) for at least 24 months.

**DETAILED DESCRIPTION OF THE INVENTION**

It has been now surprisingly found that \( T_3 \) or salts thereof is not only a physiologic \( T_3 \) catalytically, but that it may be also provided as a drug and administered in hypothyroid conditions. Accordingly, this represents the main aspect of the invention.

Furthermore, it has been found that \( T_3 \), once administered is converted to \( T_3 \) and allows maintenance of steady levels of \( T_3 \) in the body for long times (from 12 to at least 18 hrs, more preferably at least 48 hrs). This is not only particularly useful when a supplement thyroid hormone in its most active form is needed, but, once more, is completely unexpected given the rapid metabolism synthetic \( T_3 \) undergoes once administered. In this case in fact a peak level is detected in serum at about 2-3 hours and after that rapidly and completely cleared from blood within 12-24 hrs.

Furthermore, it has also been found that \( T_3 \) can be administered orally and this represents a further and particularly advantageous aspect of the invention. In fact its biological activity, measured for example by total \( T_3 \) levels in the serum of thyrectomized individuals, is detected after oral administration. This result is quite unexpected because, at variance with thyroid hormones which are not very soluble in water, \( T_3 \) or salts thereof is a polar molecule whose gastrointestinal absorption was expected to be rather inefficient. Therefore the present invention discloses that oral administration is possible and that by this route: a) \( T_3 \) is found in the
bloodstream thus demonstrating that it crosses the gastrointestinal barrier, b) is converted into the more active thyroid hormone, T₃, c) maintains T₃ levels in serum after administration for quite a long time (at least 48 hours).

[0032] According to this finding, a further object of the present invention is represented by a method for the therapeutic treatment of a hypothyroid condition, which comprises the oral administration of T₃S or the combination T₃S and T₄ as a thyroid hormone replacement therapy for a subject in need thereof. Clinical signs of hypothyroidism are the following: asthenia, fatigue, skin dryness, somnolence, speech fluency impairment, cold intolerance, weight gain and/or memory deficit. Accordingly, any of these conditions may be improved by the oral administration of T₃S alone or in combination with T₄.

[0033] In general, oral administration of T₃S and salts thereof in pharmacological compositions is proposed according to a preferred aspect of the invention for treating any hypothyroid condition or for any T₃ replacement therapy. The therapeutic treatment comprises administering compositions comprising T₃S, either alone in a dose comprised for T₃S of from 1 to 1000 μg, preferably from 2.5 to 500 μg, more preferably from 5 to 250 μg, or in combination with a second active principle, thyroxine (T₄) (combination compositions) wherein T₄ is present from 1 to 800 μg.

[0034] Preferred active principle quantities in the combination compositions are the following: T₃S 5-250 μg and T₄ 5-400 μg, T₃S 10-100 μg and T₄ 10-200 μg.

[0035] In any case, for combination compositions a preferred ratio between active principles (T₃:S:T₄) is comprised from 10:1 to 0.1:1, with a more preferred range comprised from 5:1 to 1:1. Even more preferred is the range comprised from 3:1 to 2:1 (T₃:S:T₄).

[0036] Preferred administration of the compositions is in a single daily dosage form.

[0037] Particularly preferred in the therapy of hypothyroidism, representing a further aspect of the present invention, is the association of T₃S with T₄. The hormonal association which, in theory, should more accurately mimic the normal thyroid secretion is represented by a combination of T₄ with T₃S. Actually, pharmaceutical compositions comprising both of said iodothyronines, formulated in proportions similar to the ones of the normal physiologic secretion, have already been tried and marketed. Unfortunately, the oral simultaneous administration of T₄ with T₃S was not able to reproduce the normal thyroid hormones serum levels, because of pharmacokinetics of T₄. In fact, T₄ undergoes a very quick absorption and an equally quick elimination after oral administration; its elimination rate is about 20 times higher than the one of T₃S. For this reason administration of T₄ gives raise to a dangerous peak excess in hormone concentration, if compared to the normal physiologic levels, followed by a much too fast drop to sub-physiologic levels. Thus, today most of the specialised physicians prefer using T₃S alone, even if in this way production of T₄ only depends on the perifere deiodination of T₃S, because direct secretion of T₄ by thyroid does not exist or is seriously insufficient.

[0038] On the contrary, the association of the invention avoids the above problems, because it has unexpectedly been found that, for example, after oral administration, T₃S provides T₄ serum levels that increase in a gradual way and keep steady for long periods of time, thus preventing the formation of too high peaks.

[0039] Another unexpected advantage deriving from the use of T₃S in the treatment of pathologies due to organic deficiency of T₃ consists in its recently found systemic thyrotropic activity linked to a poor inhibition of TSH secretion. This effect is particularly useful in the case of thyroidectomized patients suffering from thyroid carcinoma, when administration of T₄ must be suspended in view of carrying out radiotherapy. In such a case administration of T₃S instead of T₄ may alleviate a patient’s symptoms or disease, such as asthenia, fatigue, skin dryness, somnolence, speech fluency impairment, cold intolerance, weight gain and/or memory deficit without interfering with radioactive iodine (usually ¹³¹I) radiotherapy.

[0040] According to this observation, a further aspect of the invention relates to T₃S administration in thyroidectomized patients in case of non-surgical therapy when T₄ administration must be suspended. In fact, T₄ is usually suspended at least 40 days before radiotherapy to allow an optimal radioisotope uptake. The lack of thyroid hormones for such a long time is usually very badly tolerated by the organism which is completely depleted of thyroid hormones and begins to function within a few days, thus suffering from asthenia, fatigue, skin dryness, somnolence, speech fluency impairment, cold intolerance, weight gain or memory deficit. In contrast, T₃S, due to its low thyrotropic properties, can be administered, preferably by the oral route, up to at least 5 days, more to preferably up to at least 4, 3, or 2 days, before radiotherapy.

[0041] Another further advantage of T₃S in the therapy of hypothyroidism regards its antilumination capability. In fact, it is actively deiodinated by type I MD, which, on its part, is stimulated by thyroid hormones. In hypothyroid subjects type I MD activity is reduced, thus T₃S elimination is slowed. As a matter of fact, its effect on the body is greater. On the contrary, in case of over administration, type I MD activity is increased, thus giving more T₃S elimination, i.e. limiting possible undesired collateral effects.

[0042] Last but not least, a further advantage of T₃S is represented by the fact that it is a metabolite normally present in the body, usually non-active, i.e. non-toxic.

[0043] Accordingly, another main aspect of the present invention regards pharmaceutical formulations comprising T₃S as an active principle, as such or in combination with other thyroid hormones or pro-hormones. Particularly preferred are formulations comprising T₃S in association with T₄.

[0044] The preferred ratio of the two active principles (T₃:S) in composition comprising both active principles ranges from 10:1 to 0.1:1, with a more preferred range comprised from 5:1 to 1:1. Even more preferred is the range comprised from 3:1 to 2:1 (T₃S:T₄).

[0045] Said formulations differ in the dosage of the active principle or principles, or in the type of pharmaceutical form provided, depending on the administration route used with enteral administration being preferred.

[0046] According to this embodiment, compositions for oral administration, either liquid or solid are both suitable. Preferred liquid compositions should take into account the generally poor solubility of thyroid hormones such as T₄ and salts thereof, as well as the usually good solubility of T₃ sulphate and salts thereof. Furthermore, the use of lactose, glucose and sucrose should be avoided.

[0047] The preparation of specific pharmaceutical formulations in response to particular needs will be described in the following.
[0048] Solid Compositions.

[0049] It is known that thyroid hormones and especially levothyroxine sodium are compatible with some excipients but incompatible with others. Carbohydrates, such as starch and maltodextrin, are compatible with thyroid hormones, whereas lactose, glucose and sucrose, have been determined to be incompatible. By the use of suitable compatible diluents, glidants or lubricants and disintegrants, thyroid hormones can be formulated into tablets, capsules, or powder dosage forms.

[0050] Thus, preferred compositions of the present invention are prepared in the substantial absence of lactose, glucose, sucrose, polyvinylpyrrolidone, and/or a Poloxamer. According to this embodiment, the solid composition comprises diluents or fillers, glidants and/or lubricants and disintegrants. The compositions may also further comprise excipients, stabilizers, preservatives or dissolution enhancers.

[0051] Preferred diluents are cellulose derivatives, such as microcrystalline cellulose, powdered cellulose, silicified microcrystalline cellulose, cellulose acetate, ethyl- or methylcellulose or salts thereof. However, other diluents may be used, such as kaolin, starch and derivatives thereof, or sodium or other alkaline inorganic salts such as trisodium phosphate, tricalcium phosphate, calcium carbonate or magnesium carbonate.

[0052] Suitable disintegrants for use in the present invention include corn starch, croscarmellose and salts thereof (i.e. croscarmellose sodium) and crospovidone or salts thereof. However other disintegrants may be used such as, polyvinylpyrrolidone or croscarmellose sodium. Preferred glidants or lubricants are microcrystalline cellulose, cellulose acetate, or crospovidone.

[0053] Preferred lubricants for the present invention comprise silicones in general, including colloidal silicon dioxide, hydrous silicon dioxide, hydrated colloidal silica, such as magnesium or zinc stearate, and the preferred ones.

[0054] Suitable glidants or lubricants are chosen among magnesium stearate, talc, magnesium and zinc stearate, sodium stearate fumarate and sodium and magnesium lauryl sulphate. Preferred glidants or lubricants are magnesium stearate, talc or stearic acid.

[0055] The term glidant comprises agents working also as lubricants, and for those, such as talc, magnesium and zinc stearate or sodium dodecyl sulfate, accordingly, classification might be interchangeable.

[0056] Flavorants and colorants may be added if desired as additional optional ingredients.

[0057] Thyroid hormones, especially levothyroxine sodium and T₃S, are particularly stable in connection with cellulose derivatives. According to this embodiment solid dosage compositions with improved and superior, stability, content uniformity, good tabletting and dissolution properties which comprise T₃S or salts thereof alone or in combination with T₄S or salts thereof in the quantities above disclosed further in combination with a cellulose derivative, wherein microcrystalline cellulose or silicified microcrystalline cellulose are particularly preferred.

[0058] Thyroid hormones are preferably prepared by the synthetic route (e.g. as described for T₃S in Mol and Visser, Endocrinology 1985, 117 N. 1, 1:1-8).

[0059] In the solid composition, diluents are preferably present in a predominant amount, preferably in the range of 50 to 99.99% by weight. More preferably they are present in an amount of from 60 to 80% by weight, more preferably from 65-75% by weight.

[0060] According to a preferred embodiment, cellulose or derivatives thereof are present and preferably a second diluent is also present, preferably calcium carbonate, up to 35% of the total diluent w/w.

[0061] Preferred glidants, are selected from the group consisting of glycerol dibehenate (most preferred), talc and silica derivatives, among which magnesium trisilicate, starch or derivatives thereof, amides, tribasic calcium phosphate, are usually present in the composition in a quantity range from 1 to 10%, more preferably from 4 to 6% (w/w).

[0062] Lubricants are preferably selected in the group consisting of magnesium or zinc stearate, hydrotalcite silica and talc, more preferably magnesium stearate and hydrate colloid silica, in a total quantity of from 0.1 to 1% even more preferably the first one comprised from 0.1 to 2% and the second comprised from 0.5 to 5%.

[0063] Disintegrants for use in the present invention include starch, croscarmellose sodium and crospovidone. Preferred is croscarmellose or sodium salts thereof in a quantity ranging from 0.5 to 10% even more preferably comprised from 1-5%, most preferably comprised from 2-4%.

[0064] The moisture content of the solid dosage form, such as of a capsule or tablet, is also important. It is preferred that the moisture content is lower than 15%, even more preferably not higher than 10%. A buffer system may be present as a stabilizer in the solid dosage form.

[0065] A significant advantage of the preparations of the present invention is that they can be prepared as a direct compression formula, dry granulation formula, or as a wet granulation formula, with or without preblending of the drug, although preferably with preblending, and still achieve remarkable stability of the resulting solid dosage form preparation.

[0066] The amount of the thyroid hormone in the preparations of the present invention can vary widely, mainly depending on the administration protocol. However, due to the high potency exhibited by most of the thyroid hormones, and especially levothyroxine sodium, normally very low amounts of this thyroid hormone will be utilized.

[0067] The solid compositions comprising T₃S alone comprise T₃S of from 1 to 1000 μg; according to a further embodiment they comprise also T₄S ( thyroxine): according to this embodiment (combination compositions) T₃S is present in a quantity of from 2.5-500 μg and T₄S of from 1 to 800 μg.

[0068] Even more preferred active principle quantities in the formulation comprising two active principles are the following: T₃S 5-250 μg and T₄S 5-400 μg, T₃S 10-100 μg and T₄S 10-200 μg.

[0069] In any case, the preferred ratio between active principles (T₄S: T₃S) is comprised from 10:1 to 0:1:1, with a more preferred range comprised from 5:1 to 1:1. Even more preferred is the range comprised from 3:1 to 2:1 (T₄S:T₃S).

[0070] In the present specification, solid composition percent values refers to weight weight (w/w) ratios and the pharmaceutical dosage form is of about 50-200 mg.

[0071] The compositions of the present invention are usually prepared by blending the thyroid hormones with microcrystalline cellulose, calcium carbonate, glycerol dibehenate, croscarmellose salt, hydrate colloidal silica.

[0072] The resulting blend can be lubricated with magnesium stearate and tableted using a tablet press.
[0073] According to the invention, the solid composition of the invention are prepared in tablets and comprise either T₃, T₄ as the only active principle or T₃, T₄ in combination with a second active principle, preferably T₂₅, in the quantities and ratios above indicated, together with the following diluents, disintegrants, glidants, lubricants or excipients. In a preferred embodiment, the composition contains 1 to 1000 µg T₃, T₄ in a more preferred embodiment the compositions include 2.5 to 500 µg T₃, T₄ or more preferably 5-250 µg T₃, T₄ and the following further ingredients (in amounts described below for a 80-150 mg tablet):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount per Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium carbonate</td>
<td>20-40 mg, preferably 25-35 mg, more preferably 30 mg</td>
</tr>
<tr>
<td>Glycerol dibenenate</td>
<td>2-15 mg, preferably 4.9 mg, more preferably 5 mg</td>
</tr>
<tr>
<td>Crossmelllose sodium salt</td>
<td>1-10 mg, preferably 2-6 mg, more preferably 3.5 mg</td>
</tr>
<tr>
<td>Hydrate colloidal silica</td>
<td>0-0.5 mg, preferably 0.5-1 mg, more preferably 0.5 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.01-2 mg, preferably 0.1-1 mg, more preferably 0.5 mg</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Up to 110 mg</td>
</tr>
</tbody>
</table>

[0074] For combination compositions T₃, T₄ is preferably present in a quantity of from 2.5-500 µg and T₂₅ of from 1 to 800 µg, or, even more preferably: T₃, T₄, 5-250 µg and T₂₅, 5-400 µg, or T₃, T₄, 10-100 µg and T₂₅, 10-200 µg.

[0075] It is intended that the above quantities preferably refer to about 110 mg tablets, preferably for daily single dosage administration, even though the skilled artisan may envisage adjustments due to alternative composition forms and/or therapeutic treatment protocols. Due to the conversion rates of T₃, T₄ to T₂₅ within the body a single administration every two or three days may be also envisaged.

[0076] According to the embodiment above for tablets, the following composition for T₃, T₄ or T₂₅ and T₄ as active principle(s) (0.01-1% w/w) represents a further object of the present invention:

[0077] a diluent selected from cellulose or derivatives thereof, preferably together with a second diluent, preferably calcium carbonate, up to 35% of the total diluent (w/w);

[0078] a glidant, selected from glycerol dibenenate (most preferred), talc, silica derivatives among which magnesium trisilicate, amides, tribasic calcium phosphate, are usually present in the composition in a quantity range from 1 to 10%, most preferably 4 to 6% (w/w);

[0079] a disintegrant selected from starch, crossmelllose sodium and crospovidone. Preferred is crossmelllose sodium salt in a quantity ranging from 0.5 to 10% even more preferably comprised from 1-5%, most preferably comprised from 2 to 4% (w/w);

[0080] a lubricant selected from magnesium stearate, hydrate colloidal silica and talc, more preferably magnesium stearate and colloidal silicas, in a total quantity range comprised from 0.1 to 7% even more preferably the first one comprised from 0.1 to 2% and the second comprised from 0.5 to 5% (w/w).

[0081] Different tablet weight and active principle contents or different administration protocols may be envisaged for those skilled in the art.

[0082] The tablets according to the preferred embodiment show optimal dissolution rates (see table below) and an optimal stability of the active principle(s) (at least 24 months).

<table>
<thead>
<tr>
<th>Dissolution test</th>
<th>≥75% after 45'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>≤10%</td>
</tr>
<tr>
<td>Resistance to cracking</td>
<td>≥20N</td>
</tr>
<tr>
<td>HPLC Title T3S</td>
<td>90-110%</td>
</tr>
<tr>
<td>HPLC Title T4</td>
<td>90-110%</td>
</tr>
</tbody>
</table>

[0084] Liquid compositions, for example obtainable by crushing one or more tablets and dissolving such a mixture or the blended mixture in aqueous solutions are also possible. Optionally, trace amount (i.e. below 5%) of a pharmaceutically acceptable antioxidant is also present. It is contemplated that such compounds may include, for example, ammonium chloride and/or one or more iodide donors (e.g., sodium iodide).

[0085] The compositions of this invention may further comprise one or more physiologically acceptable formulation excipients, such as those described in "Remington, J. P., Gennaro, A. R., Remington's Pharmaceutical Sciences, Mack Publishers, Easton, (20th Edition, 2000).

[0086] The compositions of this invention are particularly suitable for oral administration.

[0087] The term “oral formulation” means that the active ingredient(s) is formulated into a product suitable for administering to an animal via the mouth. These formulations may include apart from the solid compositions described above, for example, liquids or semi-liquids, gels, pastes, oral sprays, buccal formulations, or animal feeds containing the active ingredients. Said liquid or semi-liquid compositions are typically aqueous solution.

[0088] The pharmaceutically compositions described above are prepared preferably as tablets, obtainable by direct compression of the mixture above described in powder. In some such embodiments, for example, the process further comprises combining T₃, T₄ or T₄, T₂₅ in the solid compositions described above with an aqueous composition optionally comprising a buffer for the preparation of a pharmaceutical liquid composition for oral administration.

[0089] It should be kept in mind that when the association is taken into account, the formulations of the present invention will also possibly comprise individually formulated doses of T₃, T₄, for sequential or combined administration. In this case, one suitable kit is provided, which permits administration of said active principles in ways that can differ from patient to patient, depending on the needed therapeutic application. In such a way, the specialized physician will have a wide choice of changing the prescription according to the actual need of the patient.

[0090] Just by way of a non-limitative example, in the case of oral administration, one package containing two individual blisters, which have different shape and/or color and/or different contents and/or doses, may suit the desired scope. Other possibilities exist and are easily available to the expert of the field.

[0091] The pharmaceutical compositions of the present invention are useful in the treatment of pathologies due to organic deficiency of triiodothyronine (T₃), like, for example, original hypothyroidism from autoimmune thyroid affec-
tions, hormonal production defects, thyroidecmy, congenital hypothyroidism, as well as some disorders due to reduced activity of type I 5'-iodothyronine monodeiodinase (type I MD) which is induced, for example, by hypothyroidism, non-thyroidal systemic illnesses, fast, selenium shortage and so on.

Thus, accordingly, a DTPA-T₃S monoamide (3,5-Diido-N-[[[carboxyethyl] 2-[[carboxyethyl] 2-[[bis (carboxyethyl) amino]ethyl]amino]ethyl]amine]acetyl]-O-[3-iodo-4-(sulfooxy)phenyl]-L-tyrosine) of Formula I, represents a chelating compound according to a preferred embodiment:

![Chemical Structure of Formula I]

Other molecules can be designed and synthesized by an expert in the field, through conjugation of T₃S with a variety of chelating moieties, among those suitable for complexation of lanthanide ions, e.g., nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N,N'-bis (2-hydroxynaphthoic acid) (EDDHA), ethylenediaminodisuccinic acid (EDDS), propanediaminotetraacetic acid (PDTA), diethylenetriaminopentacaetic acid (DTPA), and similar molecules. Conjugation between the chelating agent and T₃S can be obtained by a variety of methods already known to the expert in the field, including a direct amid bond formation, as exemplified in Experimental Part, or the use of bifunctional chelating agents, that may even be commercial products, such as (S)-1-p-isoniocyanatobenzylideneethyl-entriaminopentacetic acid (DTPA isothiocyanate—Invitrogen cat. 124221), or similar products.

Suitable lanthanide metals to be used as chelate labels are selected in the group consisting of: samarium, terbium, dysprosium and europium.


As an alternative embodiment, the T₃S immunoassay is developed for a particular fluorescence technique, known as DELFIA® (Dissociation Enhanced Lanthanide to Fluorescence ImmunoAssay) by which the required sensitivity is obtained. This assay, the synthesized reagents, and kits for T₃S quantitation comprising said reagents represent a further object of the invention.

A schematic of its synthesis is shown in FIG. 2 and can be summarized as follows: DTPA dihydrate is partially hydrolysed by adding an approximately equimolar amount of water dissolved in a suitable organic solvent, then the product, mainly composed of DTPA monoanhydride is reacted with T₃S, in presence of a suitable organic or inorganic base. After solvent evaporation, the oily residue is diluted with water. The
resulting precipitate is collected, washed with water and dissolved in an water/acetone mixture. This crude reaction product is purified on a column of Amberlite XAD1600, or similar resin, developing with mixtures or gradients of water/acetone. The product containing fractions are collected and evaporated to dryness, yielding the desired DTPA-T₃S monoamide.

[0999] Lanthanide complexation is obtained according to known procedures by adding an equimolar amount of a lanthanide salt to the monoamide water solution and adjusting the pH at 7 with a suitable base (e.g. NaOH). Optionally, the lanthanide chelated product can be desalted by adsorption on a resin column (e.g. Amberlite XAD1600) and elution with water/solvent mixtures.

[1000] Also in this case, a sensitivity comparable to the RIA test (see Chopra et al., ibidem) is obtained and the use of radioactive isotopes avoided this represents a clear advantage over the prior art.

[1001] According to a further embodiment, the invention comprises a kit for T₃S administration and dosage in serum, wherein said kit comprises an administration/therapeutic kit with a number of T₃S or T₄S and T₄ composition daily doses (i.e. the weekly, bi-weekly, monthly or bi-monthly need), preferably in the form of tablets as described above, and a dosage kit for T₄S immunodetection by a non-radioactive assay.

[1002] A further preferred embodiment of the kit comprises tablets with both active principles and a kit for T₃S immunodetection in serum. In a preferred embodiment the immunodetection is an ELISA test as described above.

[1003] The kit comprises a container for a non radioactive immunoassay according to the alternative embodiments described above and container for the weekly need of the solid daily dosage described above. The container for the solid daily dosage may be formulated for the weekly, bi-monthly, monthly or even multiple months needs, according to the patients and therapeutic treatment need.

**EXPERIMENTAL SECTION**

[1004] As an example, absolutely non-limiting for the skilled technican, T₃S may be administered for oral use at doses ranging from 1 to 1000 μg, preferably from 2.5 to 500 μg, more preferably from 5 to 250 μg.

[1005] Analogously, when in association with T₄S, preferred doses range from 2.5-500 μg of T₃S and 1 to 800 T₄ or, even more preferably: T₃S: 5-250 μg and T₄: 50-400 μg, or T₃S: 10-100 μg and T₄: 10-200 μg.

[1006] Two representative formulations for oral administration, selected among the preferred ones, are hereinafter enclosed by way of an example. Obviously, said formulations have no limiting effect on the other possible variations, which may also comprise different types of administration, different doses or different components depending on the specific pharmacological application or the particular pathology.

### Example A
Oral Formulation Containing T₃S

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃S</td>
<td>50 μg</td>
</tr>
<tr>
<td>Calcium phosphate dibasic anhydrous</td>
<td>103.5 mg</td>
</tr>
<tr>
<td>Mais starch</td>
<td>17.65 mg</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

### Example B
Oral Formulation Containing T₃S and T₄

#### T₃S
- Sodium carboxymethylxanthine: 5 mg
- Tale: 5 mg
- Citric acid: 2.8 mg
- Magnesium stearate: 1 mg

#### T₄
- Sodium carboxymethylxanthine: 50 μg
- T₄ sodium salt: 125 μg
- Calcium phosphate dibasic anhydrous: 103.5 mg
- Mais starch: 17.65 mg
- Microcrystalline cellulose: 5 mg
- Sodium carboxymethylxanthine: 5 mg
- Tale: 5 mg
- Citric acid: 2.8 mg
- Magnesium stearate: 1 mg

### Example C
Tablets Comprising T₃S

#### Pre Mixture
- In a 2-l. amber glass of mixer Turbola transfer a portion Avicel PH102 and a T₃S salt and mix for 5×15".

#### Final Mixture
- In a stainless steel tank of double cone mixer, transfer the pre-mixture a second portion of Avicel PH102. Mix for 10 minutes at 10 RPM.

#### Tablets
- The mix was sieved in 1-mm opening sieve and the remaining excipients: Compritol 888 ATO, Stylol 244, Aedisol, Magnesium stearate and Socal S 202 DC and the last portion of Avicel PH102 were added directly in the double cone mixer, with mixing for 20 minutes at 10 rpm.

#### Tablets
- The mixture was pressed in 110-mg tablets by a rotary tabletting machine equipped with 7-mm diameter, round, flat jewels, with one-sided break-mark in the middle.

#### Tablets were shown to have chemical and physical characteristics according to ICH Guidelines.

### Tablets were prepared and have shown to have the following characteristics:
- 20 μg dosage (25°C C₃/60%)

#### Dissolution test

<table>
<thead>
<tr>
<th>Data</th>
<th>% of Dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥75% after 45'</td>
<td>tₛ = 104.8% ( t₃ₕ = 103.3% )</td>
</tr>
<tr>
<td>% of Moisture</td>
<td>≤10%</td>
</tr>
<tr>
<td>Resistance to crushing</td>
<td>≥20N</td>
</tr>
<tr>
<td>HPLC Title T₃S</td>
<td>90-110%</td>
</tr>
<tr>
<td>HPLC Title T₄</td>
<td>90-110%</td>
</tr>
</tbody>
</table>

#### Dissolution test

<table>
<thead>
<tr>
<th>Data</th>
<th>% of Dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥75% after 45'</td>
<td>tₛ = 94.4% ( t₃ₕ = 94.2% )</td>
</tr>
</tbody>
</table>
[0115] Stability tests were carried out demonstrating that tablets are stable for at least 24 months.

Example D
Quantitation of T₃,S by Immunoassay with Chemiluminescence Detection

[0116] Synthesis of T₃,S biotin derivative

[0117] Briefly, T₃,S biotin derivative was synthesized as follows: N-hydroxy succinimidyl d-biotin-15-amido-4,7,10, 13-tetraoxapentadecylate A (50 mg; 0.0849 mmol) was solubilized in DMAC (2 mL), to which DIPEA (14.5 ul.; 0.0866 mmol) was added, while maintaining the reaction mixture under continuous stirring at 0°C. T₃,S (68.4 mg; 0.0908 mmol, prepared as described in Mol & Visser, Endocrinology 1985, 117:1-7) was then added and after a few minutes the suspension was left to heat up to room temperature to give a clear solution. It was allowed to stir for 2 h, then kept overnight at the same temperature. DMAC was evaporated under reduced pressure (10 mbar; 40°C) to give a colourless oil. The crude so obtained was dissolved in H₂O and purified by Semi-preparative HPLC. The fractions containing the product were collected, concentrated and finally lyophilized to give T₃,S-biotin as a white solid (59.6 mg; 0.0495 mmol). Yield 58%.


[0119] The assay was based on a competitive ELISA in which increasing amounts of T₃,S competed for the antibody binding with a fixed amount of T₃,S conjugated with biotin, in a white 96 well plate. The employment of the biotin-avidin interaction, which allows signal amplification, combined with luminescence as technique for signal development allowed for a sensitivity comparable to the RIA test (described in Chopra et al., J. Clin. Endocrinol. Metab., 1992, 75: 189-194).

[0120] Standard solutions of T₃,S were prepared at the following concentrations: 1000, 200, 40, 8, 1.6 pg/mL in Diluent Buffer: PBS, 0.05% Tween, 0.3% BSA

[0121] The tracer solution (T₃,S-Biotin, 180.6 μM) was prepared in the above diluent buffer. Antibody solution: T₃,S rabbit antiserum was diluted 1:50000 in Diluent Buffer plus 8 mM ANS, 1.2 mg/mL Sodium Salicylate.

[0122] A 96 well white plate was coated over night at 4°C, with 100 μL/well of 2 μg/mL anti Rabbit IgG diluted in phosphate buffer pH 7.8. At the same time, Standard solutions of biotin labelled T₃,S were combined with the diluted antiserum and the T₃,S-biotin solution as reported in Table A. The mixed samples were incubated at room temperature in the dark, over night.

[0123] The day after, the plate was washed four times with Washing Buffer (0.05% Tween 20 in PBS), then incubated in Blocking Buffer (2% BSA in Washing Buffer) for 1 h at room temperature.

[0124] Afterwards, the plate was rinsed four times with Washing Buffer, 100 μL/well of the mixed samples were added in triplicate and the plate was incubated 3 h at room temperature.

[0125] Then, the plate was rinsed three times with Washing Buffer and incubated with Streptavidin Poly-HRP (10 ng/mL in RASA, 100 μL/well) for 1 h at room temperature. After additional six washes, the plate was incubated with SuperSignal ELISA Femto Maximum Sensitivity Substrate (100 μL/well) for 5 min in the dark and the emitted light was read as counts per second (CPS) with a luminescence plate reader.

<table>
<thead>
<tr>
<th>TABLE A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration Curve Preparation</td>
</tr>
<tr>
<td>(μL)</td>
</tr>
<tr>
<td>T₃,S/1</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>CS 5 (1000 pg/mL)</td>
</tr>
<tr>
<td>CS 4 (200 pg/mL)</td>
</tr>
<tr>
<td>CS 3 (40 pg/mL)</td>
</tr>
<tr>
<td>CS 2 (8 pg/mL)</td>
</tr>
<tr>
<td>CS 1 (1.6 pg/mL)</td>
</tr>
<tr>
<td>R0</td>
</tr>
<tr>
<td>NSB</td>
</tr>
</tbody>
</table>

The calibration curve was prepared in buffer using five concentrations of the test item in the range 1.6-1000 pg/mL. The curve is shown in FIG. 1, panel a).

Example E
Quantitation of T₃,S by DELFIA®
Preparation of:
\[
\text{[3,5-Diido-N-}\{(\text{carboxymethyl})\}2-\{(\text{carboxymethyl})\}2-\{(\text{bis(carboxymethyl)}\text{ amino})\}\text{ethyl}1\text{aminio}1\text{ethyl}1\text{aminio})\text{acetyl}\}_1\text{O-}[\text{3-iodo-4-}\{(\text{sulfooxy})\text{phenyl})\cdot1\text{-tyrosinate}(6-)\text{entrope}3\text{)}\text{risodium (Formula I)}
\]

[0126]
Synthesis of Eu-DTPA-T\textsubscript{s}S monoaide


[0128] A solution of H\textsubscript{2}O (0.282 mL; 15.64 mmol) in DMAC (45 mL) was added dropwise to a suspension of N,N-bis(2,6-dioxynolen orang-4-morpholinyl)ethyl]glycine A (4.27 g; 11.94 mmol) in DMAC (85 mL) at room temperature. At the end of the addition the mixture was heated to 80°C. After 4.5 h the reaction mixture was cooled to 25°C and a solution of T\textsubscript{3}S\textsubscript{1}/5 (3 g; 5.98 mmol) and DPEA (2.71 mL; 15.92 mmol) in DMAC (85 mL) was added dropwise over 20 min DMAC was evaporated under reduced pressure (10 mmbr; 40°C). The oily residue was diluted with H\textsubscript{2}O (200 mL), obtaining precipitation of a yellowish solid that was filtered washed with H\textsubscript{2}O and dried. The crude so obtained was dissolved in Acetone/H\textsubscript{2}O 20:80 (v/v), the solution (pH 2.97) was loaded on an Amberlite® XAD-1600 resin column (200 mL; diam. 6 cm) and eluted with a Acetone/H\textsubscript{2}O gradient. The fractions containing the product having similar composition were collected and evaporated to give the ligand DTPA-T\textsubscript{s}S as a solid (1.27 g; 1.15 mmol). Yield 26%.

[0129] Europium chloride hexahydrate (0.17 g; 0.46 mmol) was added in portions to a solution of the ligand DTPA-T\textsubscript{s}S (0.51 g; 0.46 mmol) in H\textsubscript{2}O (50 mL) at 20°C (pH 2.93); after each addition the suspension was stirred until complete dissolution. Once the complexation was complete the pH was adjusted to 7.0 with 1 N NaOH and the solution was desalted by elution with water/acetone from a column of Amberlite® XAD-1600 resin (100 mL; diam. 3 cm). The fractions containing the desired product and free from salts were collected and evaporated to give the compound of Formula I (0.37 g; 0.28 mmol) a yellow solid. Yield: 61%.

[0130] The immunosassay method and solutions were as described in the Example D, with the following exceptions: a DELLFA® Wash (Perkin Elmer) was used instead of the above Washing buffer. The Tracer stock solution contained the Europium 100 µM and it was stored at 44°C, protected from light. Just before use it was diluted 1:500000 in Assay Buffer to obtain a final concentration of 440 pg/mL.

[0131] The assay was performed in Delfia Yellow plates (Perkin Elmer).

[0132] After the 3-h incubation with the mixed samples, the Formula II diluted compound solution was added (50 µL per well) to all wells. The plates were then sealed with plastic adhesive sheets and incubated under agitation for 1 h at 37°C.

[0133] After three washes, the plates were tapped dry on absorbent paper, and Delfia Enhancement Solution (Perkin Elmer) was added (200 µL). After 1 h at 25°C, the plates were read in a Victor3 instrument according to the “Europium” manufacturer protocol. A calibration curve was prepared using nine concentrations of the test item in the range 50-2000 pg/mL. The curve is shown in FIG. 1, panel b).

Example F
Clinical Trial

[0134] 1) Ethical Issues


[0136] 2) Safety

[0137] The study was designed to guarantee that plasma levels of total T\textsubscript{s}S could not exceed 196.6 ng/dl, the level obtained by the administration of the consolidated standard therapy of 20 µg T\textsubscript{s}S.

[0138] 3) Protocol

[0139] About 30 human subjects with surgically excised thyroids were administered a single dose of an oral T\textsubscript{s}S composition of the invention containing 20, 40, 80 or 160 µg T\textsubscript{s}S in tablet form. Serum levels of thyroid hormone including T\textsubscript{s}S and triiodothyronine (T\textsubscript{3}), as both free T\textsubscript{s}S (FT\textsubscript{3}) and total T\textsubscript{s}S (TT\textsubscript{s}S) were assessed by T\textsubscript{s}S RIAs, as described in Chopra et al., J. Clin. Endocrinol. Metab., 1992, 75: 189-194.

[0140] Forty eight hours prior to administration of the oral T\textsubscript{s}S composition of the invention, patients were screened for the study criteria and informed consent was requested and obtained. Twenty-four hours prior to administration of the oral T\textsubscript{s}S composition of the invention the subject was examined and all specimens for laboratory tests were collected, including thyroid function tests. On the day of the administration of the oral T\textsubscript{s}S composition of the invention, a further check of the inclusion/exclusion criteria was performed and patients were given a single dose of the oral T\textsubscript{s}S composition of the invention in tablet form according to the dose group in which they were placed.

[0141] The tablet composition was as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount per Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>T\textsubscript{s}S sodium salt</td>
<td>20.6 µg</td>
</tr>
<tr>
<td>Equivalent to T\textsubscript{s}S</td>
<td>20 µg</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>30 mg</td>
</tr>
<tr>
<td>Glycerol dibehenate</td>
<td>5 mg</td>
</tr>
<tr>
<td>Crosscarmellose sodium salt</td>
<td>3.5 mg</td>
</tr>
<tr>
<td>Hydrate celluloid silica</td>
<td>2 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>To 110 mg</td>
</tr>
</tbody>
</table>

[0142] The initial part of the study was aimed at determining the optimal dose: as none of the patients treated with the 20,40,80 and 160 µg doses of the oral T\textsubscript{s}S composition of the invention had serum levels of TT\textsubscript{s}S exceeding 196.6 ng/dl, the 160 µg dose was selected for use in the second part of the study.

[0143] 12 subjects received a single dose of the oral composition of the invention containing 160 µg T\textsubscript{s}S. The absorption of T\textsubscript{s}S was assessed by measuring the serum levels of thyroid hormones FT\textsubscript{3}, FT\textsubscript{s}S, T\textsubscript{s}S, free thyroxine (FT\textsubscript{4}) and TSH (Thyrotropin or Thyroid Stimulating hormone, “TSH”).

[0144] T\textsubscript{s}S in serum was detected with a peak level two hours after administration of the oral composition, as shown in FIG. 1. In patients lacking a thyroid there is no endogenous T\textsubscript{s}S. Thus, all T\textsubscript{s}S present in the subjects was the result of conversion of T\textsubscript{s}S from the oral compositions to T\textsubscript{s}S in vivo. By monitoring serum T\textsubscript{s}S and T\textsubscript{s}S levels after administration
of the oral T₃S compositions, it was determined that T₃S was converted to the clinically active TT₃ in a dose related fashion.

[0145] Serum levels of TSH and FT₄ were determined at 24 h and 30 minutes prior to administration, and at 24 and 48 hours. The effect of the administration of T₃S composition. Gastrointestinal absorption of T₃S was assessed by measurement of circulating serum concentrations of T₃, T₄S and FT₄. Circulating serum concentrations of FT₄ was measured pre and post dose to verify the in vivo T₃S-FT₄ conversion in patients.

[0146] Safety and tolerability were assessed by monitoring adverse events and by monitoring effects on vital signs, ECG, hematology, blood chemistry and urinalysis after administration of the oral compositions of the invention.

[0147] 4) Conclusions
[0148] Regardless of dose, the oral T₃S compositions of the invention were found to be safe and well tolerated. The mean serum concentration of T₃S (in ng/dl) for each of the four dose groups in the initial part of the study is shown in Fig. 3. For each dose group T₃S was present in the serum, with a peak level two hours after oral administration. As all subjects were thyroidectomised and thus lacking endogenous T₃S this data establishes that T₃S is absorbed from the oral compositions of the invention crosses the gastrointestinal tract and enters the bloodstream.

[0149] The mean serum concentration of T₃S and TT₃ after administration of a 160 µg dose of the oral composition of the invention is shown in Fig. 4 for a patient. TT₃ was detected within 4-5 hours of administration of the oral T₃S. As all subjects were thyroidectomised and thus lacking endogenous T₃, the only T₃ source is exogenously administered T₃S.

[0150] This data establishes that T₃S is absorbed (i.e. it crosses the Gastrointestinal Barrier) and is found in serum after oral administration, is converted to the clinically active TT₃ in a dose-related fashion and that T₃S levels in serum are still detectable 48 hrs after single dose administration.

REFERENCES


Embodiments Of The Invention


2. Triiodothyronine sulfate for use as a medicament according to embodiment 1, having thyromimetic activity.

3. Triiodothyronine sulfate according to embodiment 2, for use in the treatment of pathological due to organic deficiency of triiodothyronine.

4. Triiodothyronine sulfate according to embodiment 3, wherein said pathological comprise original hypothyroidism from autoimmune thyroid affections, hormonal production defects, thyroidectomy, congenital hypothyroidism.

5. Triiodothyronine sulfate according to embodiment 2, for use in the treatment of disorders due to reduced activity of type I 5'-iodothyronine monodeiodinase.

6. Triiodothyronine sulfate according to embodiment 3, wherein said activity of type I 5'-iodothyronine monodeiodinase comprises, among its grounds, hypothyroidism, non thyroidal systemic illnesses, fast, selenium shortage.

7. Pharmaceutical compositions comprising triiodothyronine sulfate as an active principle.

8. Pharmaceutical compositions according to embodiment 7, wherein said triiodothyronine sulfate is formulated in association with thyroxine.

9. Pharmaceutical compositions according to embodiment 7 and 8, wherein said compositions further comprise additives like excipients, diluents, solvents, carriers, dye-stuffs, flavourings, sweeteners.
10. Pharmaceutical compositions according to embodiment 7, wherein triiodothyronine sulfate is administered at doses ranging from 5 to 1000 μg.
11. Pharmaceutical compositions according to embodiment 10, wherein triiodothyronine sulfate is administered at doses ranging from 10 to 500 μg.
12. Pharmaceutical compositions according to embodiment 10, wherein triiodothyronine sulfate is administered at doses ranging from 25 to 250 μg.
13. Pharmaceutical compositions according to embodiment 8, wherein said association is administered at doses ranging from 10 to 500 μg of triiodothyronine sulfate and from 10 to 250 μg of thyroxine.
14. Pharmaceutical compositions according to embodiment 8, wherein said association is administered at doses ranging from 25 to 250 μg of triiodothyronine sulfate and from 25 to 200 μg of thyroxine.
15. Kit for the differential or sequential administration of the pharmaceutical compositions according to embodiments 8, 9 and 11 to 14.
16. Use of triiodothyronine sulfate for the preparation of the pharmaceutical compositions according to embodiments 7 to 15.

What is claimed is:
1. A solid oral dosage composition comprising TiS as the active principle, in a quantity ranging from 1 to 1000 μg and further comprising diluents, glidants or lubricants and disintegrants.
2. The solid dosage of claim 1 wherein TiS comprises 2.5 to 500 μg.
3. The solid dosage of claim 2 wherein TiS comprises 5-250 μg.
4. The solid dosage of claim 2 further comprising 5-800 μg Ti (thyroxine).
5. The solid dosage of claim 1 wherein said diluent is selected in the group consisting of: cellulose and derivatives thereof, kaolin, starch and derivatives thereof and alkaline inorganic salts.
6. The solid dosage of claim 5 wherein the cellulose derivative is selected from the group consisting of: microcrystalline cellulose, cellulose acetate or salts thereof, and ethyl cellulose or salts thereof.
7. The solid dosage of claim 5 wherein the alkaline inorganic salt is selected in the group consisting of: tri-sodium phosphate, tri-calcium phosphate, calcium sulphate, and calcium or magnesium carbonate.
8. The solid dosage of claim 1 wherein the disintegrant is selected in the group consisting of: corn starch, croscarmellose or salts thereof, crospovidone or salts thereof, polymethacrylates and maltodextrin or salts thereof.
9. The solid dosage of claim 8 wherein the disintegrant is croscarmellose or salts thereof.
10. The solid dosage of claim 1 wherein the lubricant is selected from the group consisting of: silicates, including hydrate silicon dioxide, hydrate colloid silica; magnesium stearate; zinc stearate; and talc.
11. The solid dosage of claim 1 wherein the glidant is selected in the group consisting of: glycerc dialdehyde, tribasic calcium phosphate, starch derivatives, talc, magnesium stearate and zinc stearate.
12. The solid dosage of claim 11 wherein the glidant is glycerol dibehenate or tribasic calcium phosphate.
13. The solid dosage of claim 1 in the form of a tablet.
14. The solid dosage composition of claim 1, comprising calcium carbonate, glycerol dibehenate, croscarmellose sodium salt, hydrate colloid silica, magnesium stearate and microcrystalline cellulose.
15. The solid dosage form of any one of claims 1 or 4 which comprises:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount per 80-150 mg Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium carbonate</td>
<td>20-40 mg, preferably 25-35 mg, more preferably 30 mg</td>
</tr>
<tr>
<td>Glycerol dibehenate</td>
<td>2-15 mg, preferably 4-8 mg, more preferably 5 mg</td>
</tr>
<tr>
<td>Croscarmellose sodium salt</td>
<td>1-10 mg, preferably 2-6 mg, more preferably 3.5 mg</td>
</tr>
<tr>
<td>Hydrate colloid silica</td>
<td>0.1-5 mg, preferably 0.5-4, more preferably 2 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.01-2 mg, preferably 0.1-1 mg, more preferably 0.5 mg</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Up to 110 mg</td>
</tr>
</tbody>
</table>

16. A non radioactive immunoassay for TiS quantitation comprising a non radioactive TiS-conjugate.
17. The immunoassay of claim 16 wherein the non radioactive conjugate is TiS-biotin.
18. The immunoassay of claim 17 wherein the immunoassay is a competitive ELISA.
19. The immunoassay of claim 16 wherein the non radioactive conjugate is a TiS conjugate comprising the compound of Formula I as a Lanthanide chelating agent.

20. The immunoassay of claim 16 comprising a DETAIA® assay.
21. A kit comprising a container for a non radioactive immunoassay according to claim 16 and a container for a solid oral dosage composition comprising TiS as the active principle, in a quantity ranging from 1 to 1000 μg and further comprising diluents, glidants or lubricants and disintegrants.
22. A kit comprising a container for a solid oral dosage composition according to any one of claims 2 or 4 and,

![Formula I](image)
optionally, a container for a non-radioactive immunoassay for 

t₃S quantitation comprising a non radioactive T₃S-conju-
gate.

23. A therapeutic or prophylactic treatment method for a 

hypothyroid condition due to total thyroid hormone depletion 

before ¹³¹I radiotherapy comprising administering a thy-

roidectomised patient T₃S as the sole thyroid hormone 

replacement up to 5 days before radioactive isotope admin-

istration.

24. The therapeutic treatment method of claim 23 wherein 

said hypothyroid condition is selected from the group con-

sisting of: asthenia, fatigue, skin dryness, somnolence, 

speech fluency impairment, cold intolerance, weight gain and 

memory to deficit.

25. A therapeutic treatment method for a hypothyroid con-

dition comprising administering a composition according to 

any one of claims 1, 4, 13 or 14.

26. The therapeutic treatment method of claim 25 wherein 

said hypothyroid condition is selected from the group con-

sisting of: asthenia, fatigue, skin dryness, somnolence, 

speech fluency impairment, cold intolerance, weight gain and 

memory deficit.

27. The solid dosage of claim 1 administered as a daily 

dose.