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(54) Title: STABILIZED BUDESONIDE SOLUTION AND METHOD FOR MAKING SAME

(57) Abstract: A stabilized budesonide solution and a method of forming stabilized budesonide solutions are described. The method includes sparging the budesonide solution with a non-reactive gas and, optionally, purging the storage container and/or container headspace with a non-reactive gas, protecting the solution from exposure to light, adding water to the solvent, using the S-epimer of budesonide, and/or using a concentration of budesonide of at least about 0.5%$^{w}/_{w}$ in glycol.

STABILIZED BUDESONIDE SOLUTION AND METHOD FOR MAKING SAME

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to stable budesonide solutions, the process for their preparation, and their use for producing pharmaceutical preparations.

2. Background Art

U.S. Patent No. 5,914,122 discloses a budesonide solution with a pH not exceeding 6.0 in which budesonide is dissolved in a solvent which may be water, an alcohol such as ethanol, isopropanol or propylene glycol, or a water/alcohol mixture. The '122 patent states that budesonide (16α,17 -butylidenedioxy-11β,21-dihydroxy-1,4-pregnadiene-3,20-dione) is a known active substance of the corticoid series which is employed for the treatment of inflammatory disorders such as Crohn's disease.

U.S. Patent No. 5,674,860 discloses that formoterol and/or a physiologically acceptable salt and/or solvate thereof and budesonide are used in combination for simultaneous, sequential or separate administration by inhalation in the treatment of an inflammatory respiratory disorder such as asthma. According to the '860 patent, a suitable daily dose for budesonide is 50 to 4800 μg (with a preferred dose of 100 to 1600 μg depending on the patient's age, weight, etc.). The dose can be administered by using a pressurized metered dose inhaler or as a dry powder from a dry powder inhaler.

Further discussions of budesonide can be found in U.S. Patent Nos. 4,695,625 (process of making budesonide); 5,556,964 (process of making budesonide); 5,642,728 (delivery of powdered budesonide by dry powder inhaler); and 5,932,249 (budesonide pellets with controlled release profile).

U.S. Patent No. 5,819,726 discloses a hand-held, self-contained respiratory drug dispensing device wherein the drug can be in an aqueous solution used to create an aerosol, the drug can be in a solution wherein a low-boiling point propellant is used as a solvent, or the drug can be in the form of a dry powder which is intermixed with an airflow for "particalized delivery" of the drug to a patient.

Formulation of a stable solution of budesonide for administration as a pharmaceutical preparation is desirable for treatment of asthma, Crohn's disease, and the like.

Summary of the Invention

The invention relates to stable formulations of budesonide and a method of making stable formulations of budesonide in solution.

Brief Description of the Figures

Figure 1 depicts the structures of the R and S budesonide epimers;

Figure 2 is a HPLC chromatogram obtained by assay method 1 and used to quantify budesonide epimers in propylene glycol;

Figure 3 is a graph depicting the mean percentage of budesonide remaining after storage of 0.5%$^{w}/_{w}$ budesonide in propylene glycol at room temperature (RT) and 70°C under anaerobic conditions (Expts 1A and 1B);

Figure 4 is a baseline impurity profile of budesonide (batch number NT0038) obtained by HPLC chromatogram using assay method 2;

Figure 5 is a HPLC chromatogram of a 0.8%$^{w}/_{w}$ budesonide in propylene glycol stored for 12 weeks at room temperature with continuous nitrogen purging (Expt 1C);

Figure 6 is a graph depicting the mean percentages of R and S epimers of budesonide remaining after storage of 0.5%$^{w}/_{w}$ budesonide in propylene glycol at room temperature under aerobic conditions (Expt 2A);

Figure 7 is a baseline impurity profile of budesonide (batch number NM0172) obtained by HPLC chromatogram using assay method 1;

Figure 8 is a HPLC chromatogram obtained by assay method 1 of a 0.5%$^{w}/_{w}$ budesonide in propylene glycol after 28 weeks storage at room temperature in a container with air in the headspace (Expt 2B);

Figure 9 is a HPLC chromatogram obtained by assay method 1 of a N_2 sparged 0.5%$^{w}/_{w}$ budesonide in propylene glycol after 28 weeks storage at room temperature in a sealed, N_2 purged vial (Expt 1A);

Figure 10 is a graph depicting the mean percentages of R and S epimers of budesonide remaining after storage of a 0.5%$^{w}/_{w}$ budesonide in propylene glycol at 70°C under aerobic conditions (Expt 2C);

Figure 11 is a HPLC chromatogram obtained by assay method 2 of a 0.5%$^{w}/_{w}$ budesonide in propylene glycol stored for 12 weeks at room temperature with continuous oxygen purge (Expt 2E);

Figure 12 is a graph depicting the mean percentage budesonide remaining after storage at 40°C under aerobic conditions for solutions containing a starting concentration of 0.05 or 0.5%$^{w}/_{w}$ budesonide in propylene glycol (Expts 3D and 3E);

Figure 13 is a graph depicting the mean percentage budesonide remaining after storage of 0.5%$^{w}/_{w}$ budesonide in basic or acidic propylene glycol at room temperature under aerobic conditions (Expts 4A and 5A) as compared to a control experiment of 0.5%$^{w}/_{w}$ budesonide in propylene glycol at room temperature under anaerobic conditions (Expt 1A);

Figure 14 is a graph depicting the mean percentage budesonide remaining after storage of 0.5%$^{w}/_{w}$ budesonide in basic or acidic propylene glycol at 70°C under aerobic conditions (Expts 4B and 5B) as compared to a control experiment of 0.5%$^{w}/_{w}$ budesonide in propylene glycol at 70°C under anaerobic conditions (Expt 1B);

Figure 15 is a graph depicting the mean percentages of R and S epimers of

budesonide remaining after storage of a 0.5%$^{w}/_{w}$ budesonide in basic propylene glycol at room temperature under aerobic conditions (Expt 4A);

Figure 16 is a graph depicting the mean percentages of R and S epimers of budesonide remaining after storage of a 0.5 %$^{w}/_{w}$ budesonide in basic propylene glycol at 70°C under aerobic conditions (Expt 4B);

Figure 17 is a graph depicting the percentage budesonide remaining over time following the addition of base at starting concentrations of 10^{-2}, 10^{-3}, and 10^{-4}M to a 0.5 %$^{w}/_{w}$ budesonide in propylene glycol stored at room temperature with aerobic conditions (Expts 4C-E);

Figure 18 is a graph depicting the percentage budesonide remaining over time following the addition of base at a starting concentration of 10^{-3}M to a 0.05 or 0.5%$^{w}/_{w}$ budesonide in propylene glycol continuously purged with either nitrogen or oxygen at room temperature (Expts 4F-I);

Figure 19 is a HPLC chromatogram obtained by assay method 2 of a 0.5%$^{w}/_{w}$ budesonide in propylene glycol stored under anaerobic conditions for 4 weeks at room temperature with exposure to fluorescent light (2750 Lux, which is equivalent to 2.3 million lux hours) (Expt 6);

Figure 20 is a graph depicting the mean percentage budesonide remaining after storage at 40°C under aerobic conditions of a solution containing 0.5% $^{w}/_{w}$ budesonide in propylene glycol/water (90/10 by weight) (Expt 7A) as compared to a control experiment of 0.5% $^{w}/_{w}$ budesonide in propylene glycol after storage at 40°C under aerobic conditions (Expt 3E); and

Figure 21 is a graph depicting the mean percentage budesonide remaining after storage at 40°C under aerobic conditions of a solution containing 0.05% $^{w}/_{w}$ budesonide in propylene glycol/water (50/50 by weight) (Expt 7B) as compared to a control experiment of 0.05% $^{w}/_{w}$ budesonide in propylene glycol after storage at 40°C under aerobic conditions (Expt 3D).

Detailed Description of the Preferred Embodiments

Stable formulations of budesonide in solution and a method of making the same are described herein.

Budesonide is a racemate consisting of a mixture of two epimers, 22R and 22S, which are formed by the introduction of an alkyl chain at the C_{22} atom, as illustrated in Figure 1, in roughly equivalent proportions. Both epimers have similar activities and potencies but either could potentially be employed as a separate, chemically unique drug substance. Processes for fractionating the epimers are known, for example, from EPA 92.901023.9, the disclosure of which is hereby incorporated by reference.

Because of its lipophilicity, budesonide is virtually insoluble in water but is readily soluble in alcohols, particularly polyhydric alcohols. Budesonide, up to an amount of about 1.6%$^{w}/_{w}$ solvent, can be dissolved by the use of solubilizers such as organic, water-soluble glycols. However, such solutions are too unstable for long-term pharmaceutical use because large amounts of the active substance decompose over a short time, making the solution ineffective for its intended purpose. Therefore, pharmaceutical preparations of budesonide that are available as dispersions, such as enema preparations, require mixing of budesonide, usually in powder or tablet form, with a carrier immediately before administration to the patient. The budesonide may not fully dissolve or achieve a homogenous dispersion through shaking of such a mixture, and some budesonide may be lost as granules at the bottom of the carrier, thus reducing the potency of the formulation.

Budesonide degradation in solution over time is influenced by several degradation factors. These include the presence of oxygen (oxidation), budesonide concentration, the presence of water, the acidity or alkalinity of the budesonide formulation, exposure to visible and ultraviolet light, the substance of the container, the ambient temperature during storage and the racemate composition.

A budesonide formulation as described herein has a stability such that it may be stored for at least about 12 weeks, preferably at least about 28 weeks, with

minimal degradation of budesonide. Preferably, the budesonide degradation is about 10% or less, more preferably about 5% or less, most preferably about 1% or less.

Table 1 below illustrates the solution stability of various budesonide concentrations in propylene glycol solution over time under various storage conditions, including the presence and absence of oxygen, the presence of water, increased acidity or alkalinity, exposure to visible and ultraviolet light and storage at elevated temperatures, or combinations thereof. Each line of Table 1 corresponds to data gathered during performance of the Examples set forth elsewhere herein.

It can be seen from Table 1 that the most stable solution of budesonide in propylene glycol is an anaerobic solution wherein the solution and headspace were purged with nitrogen and the solution was stored without light, at room temperature and without adjustments to the acidity or alkalinity of the solvent (see Expt. 1A). Another stable solution of budesonide further includes water as a co-solvent with the propylene glycol (see Expt. 7A).

The effect of individual degradation factors on the stability of budesonide in solution is described herein with reference to the Examples and Figures, and demonstrated by the Examples. Although budesonide in a solvent of propylene glycol is exemplified throughout, other glycols may be substituted therefor with similar effect, as would be apparent to practitioners in the art.

Table 1 Solution stability of budesonide in PG over time under various storage conditions and temperature.

Expt	Bud in PG ($\%^{w}/_{w}$)	Conditions	Container	Aerobic/ Anaerobic	Temp (°C)	Time (week)	%BUD (SD)
1A	0.5	$-O_2$	Ampule	Anaerobic	RT	28	99.0 (1.2)
1B	0.5	$-O_2$, +Temp	Ampule	Anaerobic	70	28	80.2 (3.2)
1C	0.8	$-O_2$	Gas bottle	Anaerobic	RT	12	98.8*
2A	0.5	+ O_2	Glass vial	Aerobic	RT	12	93.6 (2.6)
2B	0.5	+ O_2	Ampule	Aerobic	RT	28	96.6 (1.2)
2C	0.5	+ O_2, +Temp	Glass vial	Aerobic	70	12	34.0 (0.6)
2D	0.5	+ O_2, +Temp	Ampule	Aerobic	70	28	71.5 (2.0)
2E	0.5	+ O_2	Gas bottle	Aerobic	RT	12	97.5*
3A	0.05	$-O_2$, +Temp, [Bud]	Ampule	Anaerobic	70	4	87.7 (5.8)
3B	0.1	$-O_2$, +Temp, [Bud]	Ampule	Anaerobic	70	4	93.1 (0.6)
3C	0.5	$-O_2$, +Temp, [Bud]	Ampule	Anaerobic	70	4	100.5 (1.3)
3D	0.05	+ O_2, +Temp, [Bud]	Glass bottle	Aerobic	40	12	40.1 (1.3)
3E	0.5	+ O_2, +Temp, [Bud]	Glass bottle	Aerobic	40	12	93.0 (1.1)
4A	0.5	+[OH^-], +O_2	Glass vial	Aerobic	RT	12	90.3 (1.3)
4B	0.5	+[OH^-], +O_2, +Temp	Glass vial	Aerobic	70	12	34.4 (0.8)
4C	0.5	+10^{-2}M[OH^-], +O_2	Glass vial	Aerobic	RT	142 hr	4.19*
4D	0.5	+10^{-3}M[OH^-], +O_2	Glass vial	Aerobic	RT	142 hr	82.3*
4E	0.5	+10^{-4}M[OH^-], +O_2	Glass vial	Aerobic	RT	142 hr	98.3*
4F	0.05	+10^{-3}M[OH^-], +O_2	Gas bottle	Aerobic	RT	168 hr	33.9 (1.6)
4G	0.05	+10^{-3}M[OH^-], $-O_2$	Gas bottle	Anaerobic	RT	168 hr	29.5 (0.2)
4H	0.5	+10^{-3}M[OH^-], +O_2	Gas bottle	Aerobic	RT	172 hr	88.4*
4I	0.5	+10^{-3}M[OH^-], $-O_2$	Gas bottle	Anaerobic	RT	172 hr	73.9*
5A	0.5	+[H^+], +O_2	Glass vial	Aerobic	RT	12	84.9 (1.9)
5B	0.5	+[H^+], +O_2, +Temp	Glass vial	Aerobic	70	12	10.0 (1.3)
6	0.5	+Light, $-O_2$	Ampule	Anaerobic	RT	4	3.3*
7A	0.5	+10%H_2O, +O_2, +Temp, [Bud]	Glass bottle	Aerobic	40	12	99.0 (1.3)
7B	0.05	+50%H_2O, +O_2, +Temp, [Bud]	Glass bottle	Aerobic	40	12	61.7 (0.8)

* No standard deviation (SD) measurement was made because measurement was based on peak area.

Stable budesonide formulations are desirably formed in any suitable solvent, such as a polyhydric alcohol or glycol, more preferably propylene glycol or triethylene glycol. Other suitable solvents will be apparent to practitioners in the art. As discussed elsewhere herein, water may be added as a co-solvent to enhance the solution stability of budesonide.

Various pharmaceutical excipients may be added to a budesonide formulation as desired based on the intended use of the formulation. Such excipients and the desirable quantities thereof will be apparent to practitioners in the art based on the intended use of the formulation.

It has been discovered by the inventors herein that solutions of budesonide formulations can be provided with a high degree of stability and, thus, a longer shelf life, by removing all oxygen from the solution and storage container. Oxidation of budesonide by the action of dissolved gases in solution, air in the container, or oxidative catalysts such as metal ions greatly accelerates the rate of budesonide degradation. See Examples 1 and 2. Therefore, it is desirable to remove oxygen and oxidative catalysts from budesonide formulations in solution and storage containers for such formulations in order to minimize degradation, promote stability and, in particular, increase the shelf life of budesonide formulations in solution.

Removal of oxidative gases from budesonide formulations in solution can be achieved by sparging the solution and purging the storage container with an inert or non-reactive gas, such as nitrogen gas. In this manner, oxidative gasses such as oxygen and air are removed from the solution and/or container, thereby reducing the oxidation of the budesonide in solution.

In particular, a stable budesonide formulation in solution may be formed according to this invention by dissolving a budesonide formulation in a solution of polyhydric alcohol, preferably a glycol, more preferably propylene glycol or triethylene glycol, and sparging the solution with an inert or non-reactive gas such as nitrogen gas. The solution is preferably sparged so that all oxidative gases, particularly oxygen, are removed. Practitioners in the art will recognize that the length of time required to remove all oxygen from solution will depend on various factors, such as the oxygen content, the volume of solution, the rate at which the

sparging gas is administered, and the like. To maintain the stability of the sparged budesonide solution, the container in which the solution will be stored can also be purged with a non-reactive gas such as nitrogen gas in order to remove oxidative gases before adding the sparged budesonide solution thereto. Again, practitioners in the art will recognize that the length of time required to adequately flush any given container depends on several factors, including the size of the container, the gas used to flush the container, the flow rate of the gas, and the like.

Removal of oxidative catalysts such as metal ions from budesonide formulations, and in particular from any excipients to be added to a budesonide formulation, may be done by any method known to practitioners in the art which does not affect the stability of the budesonide formulation. For example, metals or metal ions may be removed by methods such as, but not limited to, ion exchange chromatography, as known to practitioners in the art. Optionally, chelating agents may be added to control metal ions. Such chelating agents are known to practitioners in the art and may include, for example, ethylenediaminetetracetic acid (EDTA) or ionophores, but are not limited thereto.

After filling the flushed container with the sparged budesonide solution free of oxidative catalysts, the headspace of the container is desirably flushed with an inert or non-reactive gas such as nitrogen to remove any remaining oxidative gases before sealing. If the headspace of the container is not flushed, degradation of budesonide can be expected to occur until all oxygen in the container has been used. See Examples 2A-B. After the oxygen is depleted, the budesonide solution will stabilize and not degrade further. Preferably, the headspace will be flushed until all oxidative gases are removed. It is desirable to hermetically seal the container immediately after flushing in order to prevent oxygen from re-entering the container and thus instigating budesonide degradation.

Degradation of budesonide in solution is also dependent upon the concentration of budesonide in solution. The use of larger concentrations of budesonide in the starting solution results in less total loss or degradation of budesonide by oxidation, light, heat or any other degradation factor, when compared to the starting concentration of budesonide. For example, a similarly stored 0.5 $\%^{w}/_{w}$ budesonide in propylene glycol solution is more stable than a

0.05%$^{w}/_{w}$ budesonide in propylene glycol solution, as shown in Examples 3A, C, D and E. Starting with a higher concentration of budesonide allows for a greater overall loss of budesonide by degradation before the effectiveness of the solution is impaired.

The addition of water as a co-solvent in solutions of budesonide in glycol also increases the stability of budesonide as compared to glycol solutions without water, as demonstrated in Example 7. The stabilizing effect of water is greatest when a high concentration of budesonide is employed, although budesonide is not very soluble in water. Optimum stabilization is achieved when water is present in an amount of from about 10% to about 50% by weight of the total solvent (water and glycol). The use of water in amounts greater than about 50% by weight diminishes the solubility of budesonide, thus decreasing stabilization of the budesonide formulation. The use of water in amounts less than about 10% by weight has negligible influence on the stability of the budesonide formulation. While not wishing to be bound by theory, the inventors herein propose that the addition of water as a co-solvent effectively increases the ratio of budesonide to glycol, thus effectively increasing the concentration of budesonide in the glycol solvent and reaping the beneficial effects of a higher budesonide concentration, as discussed elsewhere herein, including a lowered apparent oxidative degradation effect.

Budesonide solution stability is further affected by increases in both alkalinity and acidity of the solvent, as shown in Examples 4 and 5. Corresponding examples were performed and the results reported in *Pharm. Sci.* (1998) 1 (1:suppl) S-307, incorporated herein in its entirety by reference. As seen from Examples 4 and 5, increases in alkalinity or acidity of the solvent decrease the stability of the budesonide solution. Thus, it is desirable that the apparent pH of the chosen solute is not altered. In particular, as can be seen in the results set forth in *Pharm. Sci.* (1998) 1 (1:suppl) S-307, an apparent pH of about 5.7, achieved with the use of propylene glycol as a solvent, achieves good stability as compared to an apparent pH of either 11.0 or 0.5 in the presence of oxygen.

The container used to store the budesonide solution is preferably non-reactive with budesonide or any other component of the solution. Certain

materials may influence the alkalinity or acidity of materials held therein. Thus, the apparent alkalinity [OH^-] or acidity [H^+] of a container may affect the rate of budesonide degradation. For example, materials such as Type II and III glass which behave as alkali donors, could accelerate budesonide degradation. Thus, the material used to contain the budesonide solution can be selected on the basis of materials that are non-reactive with budesonide and other components of the solution. Such containers may include, for example, Type I glass and certain non-reactive polymers, but are not limited thereto. Suitable container materials will be apparent to practitioners in the art.

Budesonide degradation is accelerated by exposure to visible and ultraviolet light (UV/VIS), as exemplified by Example 6. UV/VIS promotes photolytic catalysis of degradation processes. Stable solution formulations of budesonide can be produced using commercially available light resistant containers. For example, the solution container itself may be light resistant, comprising amber glass or other materials known to practitioners in the art. Alternatively, or in addition thereto, the container for the budesonide solution may be packaged in light resistant materials, such as, but not limited to, foil, cardboard, opaque plastic or the like, as known to practitioners in the art.

Another destabilizing factor is the mixture of racemates found in budesonide formulations. The S-epimer has been found by the inventors herein to be more stable than the R-epimer of budesonide under destabilizing conditions such as increased temperature, the presence of oxygen, and basic solution conditions, taken alone or in combination (see Figs. 10, 15 and 16 and Examples 2C and 4A). Thus, solutions comprising racemic mixtures of budesonide wherein the S-epimer predominates, and pure S-epimer formulations of budesonide have greater stability than R-epimer or primarily R-epimer formulations of budesonide when exposed to degradation factors.

Further, as demonstrated throughout the Examples, temperature is a degradation factor of budesonide. In particular, exposure of budesonide formulations to high temperatures of 40°C or greater, particularly 70°C or greater, greatly increases budesonide degradation. See, for example, Examples 1A and B, 2A-D, 4A and 5A.

Various degradation factors have been discussed individually herein. However, as presented, many of these factors may act in combination, or be interrelated, in producing or accelerating degradation of a budesonide formulation. Knowledge of these factors and the various interrelations between them aids in making an extremely stable budesonide formulation. In particular, stability of a budesonide formulation can be achieved or enhanced by any one or more of the following: removal of oxygen and/or oxidative substances from the budesonide formulation, container and/or container headspace; increasing the budesonide concentration; adding water as a co-solvent (which effectively increases budesonide concentration); maintaining the effective pH of the solvent, particularly an effective pH of about 5.7; use of containers which do not affect the apparent alkalinity or acidity of the budesonide formulation therein; removal of light, both ultraviolet and visible; increasing the ratio of S-epimer to R-epimer; and maintaining temperatures of the stored solution at about room temperature.

The embodiments described herein so far have exemplified nitrogen for sparging the budesonide solution and flushing the container and headspace. However, other gases which are unreactive with budesonide and, preferably, other components of the solution, may also be used. For example, inert gasses such as the noble gases, for example argon, may be used, so long as traces of such gases in the solution will not be detrimental to the patient or react with any component of the pharmaceutical composition of budesonide.

Examples demonstrating degradation and stability of budesonide solutions over time with exposure to oxygen, varying budesonide concentration, the presence of water, increased alkalinity or acidity, light, increased temperatures and varying racemate mixtures are set forth herein.

Examples

Solution formulations of budesonide were subjected to a series of stability stress tests. In the tests, the solution formulations were subjected to one or more "stressors" or degradation factors including anaerobic conditions, wherein the solutions, container and container head space were purged with nitrogen; aerobic conditions wherein one or more of the solution, container or container headspace

contained oxygen; heat; changes in alkalinity or acidity of the solution; UV/VIS light; water as a co-solvent; and changes in budesonide concentration.

In the following examples, the materials used, unless otherwise specified, were as follows:

budesonide (EP grade), from Spectrum Quality Products;
propylene glycol (USP), from Fisher Scientific Co.;
deionized water;
nitrogen, from BOC Gases;
oxygen, from BOC Gases;
hydrochloric acid, from VWR Scientific Inc.; and
sodium hydroxide (1N), from Fisher Scientific Co.

Abbreviations as used herein are as follows:

BUD - budesonide
conc - concentration
HCl - hydrochloric acid
HPLC - high performance liquid chromatography
M - molar
ml - milliliter
n - number of samples tested
NaOH - sodium hydroxide
PG - propylene glycol
SD - standard deviation
μg - microgram
μl - microliter
μm - micrometer
$^{v}/_{v}$ - volume per volume
$^{w}/_{w}$ - weight per weight
%DFN - percent Difference from Nominal concentration
%RSD - percent Relative Standard Deviation

Other abbreviations are meant to have the meaning commonly known in the art unless otherwise indicated.

Budesonide Quantification Methods

All budesonide solutions were assayed by HPLC at designated time intervals in order to quantify the amount of budesonide remaining and the R/S epimer ratio. For each experiment, the budesonide in propylene glycol

formulation was diluted with the appropriate mobile phase (based on the assay method to be used) to produce an approximate 10 μg/ml solution, which was then assayed with the appropriate assay method (see assay methods 1 and 2 below). A typical chromatogram using assay method 1 is shown in Figure 2. In the following test results, budesonide was quantified as a percentage of its content at time=0. Statistical comparisons were made using a t-test where appropriate.

HPLC assays 1 and 2 were conducted as described below.

Assay 1: HPLC was used to quantify budesonide epimers in PG. The apparatus was set up as follows:

Column: Hypersil® Elite C18 column, 5μm, 4.6mm x 250mm
Mobile Phase: 43:57 reagent alcohol:water
Flow rate: 1ml/min.
Wavelength (λ): 240nm
Injection Volume: 20 μl in mobile phase

Assay 2: This method provided an improved separation of budesonide degradation products and enabled validation of the quantification results obtained in assay method 1. The apparatus was set up as follows:

Column: Hypersil® Elite C18 column, 5μm, 4.6mm x 150mm
Mobile Phase: 2:30:68 reagent alcohol:acetonitrile:phosphate buffer pH 3.4
Flow rate: 1.5 ml/min.
Wavelength (λ): 240nm
Injection Volume: 20 μl in mobile phase

Standard samples of budesonide batch numbers NT0038 and NM0172 were produced for each assay method by dissolving budesonide in the mobile phase for that method in an amount of 500μg/ml. These samples were used as standards and for assay validation. The HPLC chromatogram of budesonide batch number NT0038 by assay method 2 in shown in Figure 4, and the HPLC chromatogram of budesonide batch number NM0172 by assay method 1 is shown in Figure 7.

Assay validation: Details of the assay validation for assay methods 1 and 2 are

shown in Tables 2 and 3 below. Calibration curves were constructed for both assay methods (not shown) and were observed to be linear over the range of 2.5 μg/ml to 25 μg/ml (r^2 = 0.999). Validation data revealed that the assays had linear UV detector response, good system precision and accuracy, and high sensitivity and resolution for budesonide, its degradation products and impurities. The methods were capable of separating the impurities and degradation products of budesonide successfully in both standard and solution stability samples.

Table 2. Mean precision and accuracy data for reference standard samples of budesonide measured using assays 1 and 2 (n=5).

Assay Method #	Nominal conc.	Mean measured conc. (%DFN)	Precision Within day (%RSD)	Precision Between day (%RSD)
1	5.5 μg/mL	5.5 μg/mL(-0.4%)	0.6 %	1.0 %
2	10.3 μg/mL	10.4 μg/mL (1.3%)	1.1 %	1.6 %

Table 3. Mean accuracy data for the sampling procedure of budesonide in PG solutions using assays 1 and 2 (n=5).

Assay Method #	Bud in PG nominal conc.	Mean measured conc (% DFN)
1	0.475%$^{w}/_{w}$	0.475%$^{w}/_{w}$ (-0.06%)
2	0.494%$^{w}/_{w}$	0.491%$^{w}/_{w}$ (-0.59 %)

In each of the following examples, the budesonide solutions were stored in a gas bottle, a glass vial, an ampule or a glass bottle for the duration of the experiment. Unless defined otherwise, the storage conditions for each container are as follows:

gas bottle: a 250 ml unsealed glass bottle protected from light wherein the solution therein is continuously purged with nitrogen or oxygen;

glass vial: a 10 ml glass non-airtight vial protected from light that is periodically opened for sampling and allowed replenishment of environmental air;

ampule: a 2 ml sealed amber colored Type 1 glass vessel (protected from light) with purged headspace of nitrogen or

oxygen; and

glass bottle: a 100 ml sealed amber colored bottle wherein the headspace is purged with nitrogen or oxygen each day to maintain constant environmental conditions.

1. Absence and Presence of Oxygen

Example 1: Stabilization using anaerobic storage conditions

Methods: The following anaerobic stability experiments were performed using solutions of budesonide in propylene glycol.

Expt. 1A. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at room temperature in sealed amber colored ampules. The solution and vial headspace were purged with nitrogen to produce anaerobic conditions. The solution was protected from exposure to light and moisture ingress. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). The test solution was then nitrogen purged and 1 ml was placed into amber colored ampules (2ml). The headspace of the ampules was purged with nitrogen. The ampules were then sealed and stored at room temperature without light. Samples were taken and assayed at time = 0, 4, 8, 12, 28 weeks. Results are shown in Table 4.

Expt. 1B. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at 70°C in sealed amber colored ampules. The solution and vial headspace were purged with nitrogen to produce anaerobic conditions. The solution was protected from exposure to light and moisture ingress. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). The test solution was then nitrogen purged and 1 ml was placed into amber colored ampules (2ml), The headspace of the ampules was purged with nitrogen. The ampules were then sealed and stored at 70°C. Samples were taken and assayed at time = 0, 4, 8, 12, 28 weeks. Results are shown in Table 4.

Expt. 1C. 0.8%$^{w}/_{w}$ budesonide (batch number NT0038) in propylene glycol was stored at room temperature in a glass gas bottle. The solution was continuously purged with flowing nitrogen gas and protected from exposure to light. Immediately following preparation, the test solution was assayed for budesonide content (by comparative peak area) and epimer ratio using assay method 2 (time=0). The test solution was placed in a 250 ml glass gas bottle and nitrogen gas was continuously purged through the solution while stored at room temperature. Samples were taken and assayed at time = 0, 2, 4, 8, and 12 weeks. Results at time = 0 and time = 12 weeks are shown in Figures 4 and 5, respectively.

Results: Solutions of budesonide in PG stored under anaerobic conditions and protected from light were stable at room temperature for a period of 28 weeks. Experiment 1A revealed that the mean (SD) percentage budesonide remaining following storage at room temperature was 99.0 (1.2) % (Table 4 and Figure 3). There was no statistically significant difference between the starting concentration and the measured concentration after anaerobic storage at room temperature for 28 weeks. In addition, there was no change in the R and S epimer ratio over this time period. Exposure to elevated temperature (70°C, Expt. 1B) accelerated the degradation of budesonide stored under anaerobic conditions (see Table 4 and Figure 3). The mean (SD) percentage budesonide remaining was 80.2 (3.2)% of the initial concentration. The ratio of the R and S epimers remained unchanged following storage at 70°C for 28 weeks.

Those skilled in the art will recognize that while pharmaceutical stability testing often occurs under accelerated conditions, 70°C is an extreme temperature which is much higher than any storage temperature used in practice. Moreover, a formulation found to be stable under these conditions would be acceptable as "stable" under those more commonly used in the industry.

Table 4 Mean (SD) percentage budesonide remaining of a 0.5%$^{w}/_{w}$ budesonide in propylene glycol following anaerobic storage at room temperature and 70°C (Assayed using Method 1)

Time (weeks)	Expt. 1A Room Temperature % BUD remaining (SD)	Expt. 1B 70°C % BUD remaining (SD)
0	100.0 (1.1)	100.0 (1.1)
4	98.2 (1.70)	96.5 (1.30)
8	98.3 (0.5)	89.0 (0.6)
12	98.7 (1.0)	87.4 (1.9)
28	99.0 (1.2)	80.2 (3.2)

Experiment 1C used a different chromatographic technique to monitor room temperature degradation of budesonide in propylene glycol solutions when exposed to a continuous nitrogen purge. Figure 4 is a chromatogram showing the impurity profile of the budesonide (batch number NT0038) used in this study. The measured purity of this batch of budesonide was 98.7% (as measured by peak area determination). Two main impurity peaks were observed and labeled as 1 and 2, respectively. This is the baseline chromatographic profile for this stability study.

Figure 5 shows a chromatogram of the budesonide in propylene glycol solution stored for 12 weeks at room temperature while being continuously purged with nitrogen. The measured amount of budesonide in this sample, based upon peak area determination, was 98.8 %. The profile is essentially unchanged from the baseline profile (Figure 4). Additional peaks were observed with retention times of 2.01 minutes (approximately 0.04 % of total peak area) and 12.25 minutes (approximately 0.05% of total peak area), respectively, however, neither peak exceeded the pharmaceutically significant threshold (for identification) of 0.1%. This chromatogram confirmed that solutions of budesonide in propylene glycol were stable when stored at room temperature following a nitrogen purge.

Example 2: Effects of Aerobic Conditions

Methods: The following series of aerobic stability experiments were performed using solutions of budesonide in propylene glycol to demonstrate the effect of oxygen on the solution stability of budesonide.

Expt. 2A. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at room temperature in clear glass non-airtight vials which were periodically opened for sampling and allowed replenishment of environmental air. The solution was protected from light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at time = 0, 2, 4, 12 weeks.

Expt. 2B. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at room temperature in amber colored sealed ampules with a limited air headspace. The solution was protected from exposure to light and moisture ingress. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at time = 0, 4, 8, 12, 28 weeks.

Expt. 2C. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at 70°C in clear glass non-airtight vials which were periodically opened for sampling and allowed replenishment of environmental air. The solution was protected from light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at time = 0, 2, 4, 12 weeks.

Expt. 2D. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at 70°C in amber colored sealed ampules with a limited air headspace. The solution was protected from exposure to light and moisture ingress. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at time = 0, 4, 8, 12, 28 weeks.

Expt. 2E 0.5%$^{w}/_{w}$ budesonide (batch number NT0038) in propylene glycol was stored at room temperature in a glass gas bottle. The solution was continuously purged with flowing oxygen gas. The solution was protected from exposure to light. Immediately following preparation, the test solution

was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at time = 0, 2, 4, 8, 12 weeks.

Results

1. Room Temperature Storage: Table 5 shows the mean (SD) percentage budesonide remaining and the presence of additional degradation product peaks following storage of each sample at room temperature. For comparative purposes, the results from a similar anaerobic study (Expt 1A) are also shown. Degradation was observed (decrease in % budesonide remaining or presence of degradation peaks) for solutions of budesonide in propylene glycol stored at room temperature in non-airtight vials for 12 weeks (Expt 2A). These samples were exposed to an unlimited amount of atmospheric air (approximate oxygen concentration in air = 21%). Degradation peaks are observed in addition to the epimer peaks. Figure 6 reveals that there was no significant difference in the R and S epimer ratio following storage at room temperature as compared to the initial ratio.

Samples having a limited air headspace stored for 12 weeks at room temperature (Expt 2B) did not exhibit significant degradation. However, degradation was evident after storage for 28 weeks. Figure 7 is a chromatogram showing the impurity profile of budesonide (batch number NM0172) used in this study. The measured purity of this batch of budesonide was 99.5 % (as measured by peak area determination). Five main impurity peaks are observed and labeled as 1-5, respectively. This is the baseline chromatographic profile for this stability study assayed using assay method 1.

Figure 8 shows the HPLC chromatogram for a sample having a limited air headspace stored for 28 weeks at room temperature. There were significant additional degradation peaks shown in Figure 8 compared to the baseline sample (Figure 7). In Figure 8, there were 14 peaks, each having a relative peak area greater than 0.1 % of the total peak area. A degradation peak area greater than 0.1 % is conventionally found to be significant to the pharmaceutical industry and requires identification and limitation.

In contrast, solutions of budesonide in propylene glycol sparged with nitrogen prior to loading into sample containers were observed to be stable at 28

weeks (Expt. 1A). The sample containers were purged with nitrogen prior to the addition of the nitrogen sparged solution, then the headspace above the solution was purged with nitrogen and the containers were sealed. Room temperature storage of these samples revealed no degradation after 28 weeks. Figure 9 shows a chromatogram of nitrogen sparged budesonide in propylene glycol solution stored for 28 weeks at room temperature in a nitrogen purged sealed vial (N_2 only). The profile is essentially unchanged from the baseline profile (Figure 7). An additional peak is observed with a retention time of 30.5 minutes (approximately 0.2 % of total peak area). While not wishing to be bound by theory, it is believed this peak is a degradation product of budesonide or one of the starting impurities (e.g. impurity peak 3, whose relative peak area has decreased from 0.11% to 0.03%), or a combination of both. Figure 9 and Table 5 confirm the room temperature stability of budesonide in propylene glycol solutions after sparging the solution and purging the container with nitrogen prior to stability testing. There was no statistical difference between the initial budesonide content and the percentage budesonide remaining after 28 weeks storage for the nitrogen purged vials (99.0%).

Table 5 Mean (SD) percentage budesonide remaining (% BUD) and presence (+) or absence (-) of degradation peaks following storage at room temperature.

Sample description	Storage time	% BUD remaining	Additional peaks
Unsealed vial (unlimited air) - Expt 2A	12 weeks	93.6(2.6) %	+
Sealed vial (with air headspace) - Expt 2B	12 weeks	*98.0(2.1) %	-
Sealed vial (with air headspace) - Expt 2B	28 weeks	96.6 (1.2) %	
Nitrogen sparged vial (N_2 only) - Expt 1A	28 weeks	*99.0 (1.2) %	-

* not statistically different from starting concentration

2. 70°C storage: Table 6 reveals the results of studies performed under elevated temperature conditions (70°C). Following storage for 12 weeks at elevated temperature conditions with a replenishable air supply, budesonide degraded to 34.0% of its starting amount. In the case of samples with a limited headspace,

degradation plateaus after about 12 weeks, indicating the exhaustion of a reactive gaseous component, presumably oxygen, in the PG solutions. In comparison, storage at 70°C of the nitrogen sparged budesonide solution in a purged, sealed vial with a nitrogen purged headspace revealed slight degradation of budesonide, but significant retardation of degradation in comparison to the other tested storage formulations under these elevated temperature conditions.

Figure 10 reveals a change in the measured R and S epimer ratio after storage at 70°C under aerobic conditions in glass vials as compared to the initial epimer ratio. Following storage at 70°C, the S-epimer was observed to be more stable than the R-epimer, and the R and S epimer ratio changed from an initial value of 57.4/42.6 to 51.4/48.6 after 12 weeks (Figure 10: * indicates $P < 0.05$; (t-test) with respect to the value at time zero).

Table 6 Mean (SD) percentage budesonide remaining (% BUD) and presence (+) or absence (-) of degradation peaks following storage at 70°C.

Sample description	Storage time	% BUD remaining	Additional peaks
Unsealed vial (unlimited air) - Expt 2C	12 weeks	34.0 (0.6) %	+
Sealed vial (with air headspace) - Expt 2D	12 weeks	72.2 (2.7) %	+
Sealed vial (with air headspace) - Expt 2D	28 weeks	71.5 (2.0) %	+
Nitrogen sparged vial (N_2 only) - Expt 1B	28 weeks	80.2 (3.2) %	+

As demonstrated by the above experiments, budesonide degradation in propylene glycol is related to the oxygen content of the solution and its storage environment. Sparging of the solution and purging of the container and headspace with nitrogen significantly reduces degradation and degradation rates, even at elevated temperatures. Limitation of oxygen availability also reduces overall drug degradation.

Experiment 2E was performed at room temperature using $0.5\%^{w}/_{w}$ budesonide in propylene glycol and was continuously purged with oxygen. This is directly comparable with experiment 1C (room temperature continuously purged with nitrogen). These experiments were performed to verify that: (1) the presence of oxygen was a destabilizing reactant in solution and that (2) the removal of oxygen during a nitrogen purge and addition of nitrogen produces a stable

solution. The HPLC assay methodology employed was assay method 2.

Figure 11 confirms that the presence of oxygen in solution significantly accelerates the degradation of budesonide in propylene glycol solutions. The measured amount of budesonide in experiment 2E, based upon peak area determination, was 97.5 % following storage for 12 weeks compared to 98.8% for nitrogen purged samples (Expt. 1C) as shown in Figure 5. For experiment 2E, which was stored at room temperature with a continuous oxygen purge, the chromatographic profile reveals the presence of numerous additional degradation peaks compared to the baseline sample and the nitrogen purged sample (Expt. 1C) (see Figures 4 and 5).

2. Concentration of Budesonide

Example 3: Effects of Budesonide Concentration

Methods: The following stability experiments were performed using solutions of differing budesonide concentration in propylene glycol.

Expts. 3A-C. 0.05 **(3A)**, 0.1 **(3B)** and 0.5%$^{w}/_{w}$ **(3C)** budesonide (batch number NT0038) in propylene glycol were prepared and stored at 70°C in sealed amber colored ampules (Type I glass). The solution and vial headspace were purged with nitrogen to produce anaerobic conditions. The solution was protected from exposure to light and moisture ingress. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0). Samples were taken and assayed at time = 0, 1, 2, 4 weeks.

Expts. 3D-E. 0.05 **(3D)** and 0.5%$^{w}/_{w}$ **(3E)** budesonide (batch number NT0038) in propylene glycol were prepared and stored at 40°C in 100 ml sealed amber colored bottles. The solution and headspace of the bottles were purged with oxygen each day in order to maintain constant environmental conditions. The solution was protected from exposure to light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0). Samples were taken and

assayed at time = 0, 2, 4, 6, 8, 10, 12 weeks.

Results: Experiments 3A-C showed that budesonide degradation, although minimal under anaerobic conditions, was further reduced by increasing the solute (budesonide) starting concentration. Table 7 shows the mean (SD) percentage budesonide remaining for solutions containing 0.05, 0.1 and 0.5%$^{w}/_{w}$ budesonide in propylene glycol stored under anaerobic conditions at an elevated temperature to maximize degradation. After 4 weeks, there was no significant change in the measured concentration of the 0.5%$^{w}/_{w}$ budesonide in propylene glycol. In contrast, the 0.05%$^{w}/_{w}$ budesonide in propylene glycol solution had a mean (SD) percentage budesonide remaining of 87.7 (5.8)% ($P<0.05$; t-test).

Table 7 Mean (SD) percentage budesonide remaining for solutions containing 0.05, 0.1 and 0.5 %$^{w}/_{w}$ budesonide in propylene glycol, respectively, following anaerobic storage at 70°C (Assayed using Method 2)

Time (weeks)	0.05%$^{w}/_{w}$ BUD in PG % BUD remaining (SD)	0.1%$^{w}/_{w}$ BUD in PG % BUD remaining (SD)	0.5%$^{w}/_{w}$ BUD in PG % BUD remaining (SD)
0	100.0 (0.6) %	100.0 (1.3) %	100.0 (1.0)
1	94.4 (0.5) %	95.4 (0.8) %	N/A
2	89.8 (1.8) %	93.4 (1.8) %	98.9 (0.6)
4	87.7 (5.8) %	93.1 (0.6) %	100.5 (1.3)

Experiments 3D and 3E showed a similar concentration effect on solute stability, as described above, wherein an increase in budesonide starting concentration reduced the rate of budesonide degradation. However, degradation was accelerated even at the moderate temperature conditions (40°C) due to the presence of oxygen. Table 8 and Figure 12 clearly demonstrate a significant difference in the stability of budesonide at starting concentrations of 0.05 and 0.5 %$^{w}/_{w}$ budesonide in propylene glycol. Table 8 shows the mean (SD) percentage budesonide remaining for solutions containing 0.05 and 0.5 %$^{w}/_{w}$ budesonide in propylene glycol stored under

aerobic conditions at 40°C. After 12 weeks, the mean (SD) percentage budesonide remaining was 93.0 (1.1) % and 40.1 (1.3) %, respectively, for the 0.5 % $^{w}/_{w}$ and 0.05 $\%^{w}/_{w}$ budesonide in propylene glycol.

A similar effect was observed when these experiments were repeated at room temperature, confirming the significant effect of budesonide starting concentration on budesonide solution stability in propylene glycol.

Table 8 Mean (SD) percentage budesonide remaining for solutions containing 0.05 and 0.5 % $^{w}/_{w}$ budesonide in propylene glycol, respectively, following aerobic storage at 40°C (Assayed using Method 2)

Time (weeks)	0.05 % $^{w}/_{w}$ BUD in PG % BUD remaining (SD)	0.5 % $^{w}/_{w}$ BUD in PG % BUD remaining (SD)
0	100.0 (0.6) %	100.0 (0.5) %
2	72.4 (0.6) %	98.0 (0.6) %
4	63.5 (0.8) %	98.0 (0.8) %
6	55.5 (0.6) %	95.7 (1.9) %
8	50.6 (1.1) %	94.7 (1.1) %
10	46.1 (0.7) %	93.1 (2.4) %
12	40.1 (1.3) %	93.0 (1.1) %

3. Alkalinity and Acidity

Example 4: Effects of Alkalinity

Methods: The following series of stability experiments were performed using solutions of budesonide in propylene glycol with the addition of controlled amounts of base to alter solution alkalinity.

Expts. 4A-B. 0.5$\%^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at room temperature **(4A)** and 70°C **(4B)**, respectively, in clear glass non-airtight vials which were periodically opened for sampling and allowed replenishment of environmental air. Sodium hydroxide was added to the solution to produce an apparent pH of 11 and a starting [OH^-] of 10^{-3}M. The solutions were protected from light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at

time = 0, 2, 4, 12 weeks.

Expts. 4C-E. 0.5%$^{w}/_{w}$ budesonide (batch number NT0038) in propylene glycol was stored at room temperature in clear glass non-airtight vials which were periodically opened for sampling and allowed replenishment of environmental air. Sodium hydroxide was added to produce solutions with starting [OH^-] of 10^{-2}M (**4C**), 10^{-3}M (**4D**) and 10^{-4}M (**4E**), respectively. These solutions were protected from light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0). Samples were taken and assayed at appropriate time intervals dependent upon the [OH^-].

Expts. 4F-I 0.05 and 0.5%$^{w}/_{w}$ budesonide (batch number NT0038) in propylene glycol were stored at room temperature in a glass gas bottle. Solutions having a starting [OH^-] of 10^{-3}M were continuously purged with flowing oxygen (**4F** and **4H**, respectively) or nitrogen (**4G** and **4I**, respectively) gas. These solutions were protected from exposure to light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0). Samples were taken and assayed at appropriate time intervals dependent upon the storage conditions.

Results: Experiments 4A and 4B revealed that following storage for 12 weeks at room temperature and 70°C, there was significant budesonide degradation in basic propylene glycol solutions. Figure 13 shows the mean (SD) percentage budesonide remaining following storage at room temperature (also shown for reference is a control experiment under anaerobic condition, Expt 1A). Figure 14 shows the mean (SD) percentage budesonide remaining following 12 weeks of storage at 70°C (also shown for reference is a control experiment under anaerobic condition, Expt 1B). Figures 13 and 14 have error bars showing standard deviations (n=3) and demonstrate that stress conditions of alkalinity accelerate budesonide

degradation when stored under aerobic conditions.

Further, immediately following the addition of alkali to the budesonide in PG solution, a significant change in the R and S epimer ratio was observed (Figure 15). The R and S epimer ratio changed from 57.4/42.6 to 55.8/44.2. After 12 weeks at room temperature under alkaline conditions, the R/S epimer ratio became 51.8/48.2. A similar pattern of R/S epimer ratio inversion was observed at 70°C (Figure 16). Thus, under basic solution conditions, the S-epimer was more stable than the R-epimer. (In Figures 15 and 16, * indicates $P < 0.05$ (t-test) with respect to the value at time zero.) The above results demonstrate that the R- and S-epimers have different stability profiles, with the S-epimer demonstrating greater stability in propylene glycol at 70°C or under basic solution conditions than the R-epimer.

Figure 17 shows the budesonide degradation profiles as a function of increasing base concentration when stored under aerobic conditions (Expts. 4C-E). The rate of budesonide degradation was rapid for the solution containing base at a concentration of 10^{-2}M, with the percentage budesonide remaining (calculated by comparative peak area) following storage for 142 hours being 4.19 %. For the solutions containing base concentrations of 10^{-3}M and 10^{-4}M, the percentage budesonide remaining was 82.3 % and 98.3 %, respectively, at the same time point. Budesonide degradation at room temperature was observed to increase as a function of increasing base concentration (Figure 17).

Experiments 4F-I showed that the degradation profiles of solutions with the same initial base concentration (10^{-3}M) were significantly different dependent upon the presence or absence of oxygen (see example 2) and the initial budesonide concentration (see example 3). Figure 18 summarizes the mean percentage budesonide remaining as observed in these experiments. Base catalyzed degradation of budesonide in the presence of oxygen was rapid, and the extent of degradation was dependent upon the initial starting concentration of budesonide. In contrast, anaerobic degradation rates were slower.

The aerobic experiments 4F and 4H appeared to plateau in Figure 18. While not wishing to be bound by theory, this may indicate the exhaustion of the base catalyst. In contrast, reflecting the slow rates of degradation under anaerobic conditions, plateaus were not observed over the duration of this study for anaerobic experiments 4G and 4I. The experiments reflected the effects of initial budesonide concentration and aerobic/anaerobic conditions, using the addition of base as a degradation catalyst.

While not wishing to be bound by theory, it appears that the effects of a basic solution outweigh the effects of an aerobic or anaerobic environment for higher budesonide concentrations. Further study of the interplay of alkalinity and aerobic/anaerobic effect is therefore warranted.

Example 5: Effects of Acidity

Methods: The following series of stability experiments were performed using solutions of budesonide in propylene glycol with the addition of controlled amounts of acid to alter solution acidity.

Expt. 5A-B. 0.5%$^{w}/_{w}$ budesonide (batch number NM0172) in propylene glycol was stored at room temperature **(5A)** and 70°C **(5B)**, respectively, in clear glass non-airtight vials which were periodically opened for sampling and allowed replenishment of environmental air. Hydrochloric acid was added to the solution to produce an apparent pH of 0.5 and a starting $[H^+]$ of $3x10^{-1}$M. These solutions were protected from light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 1 (time=0). Samples were taken and assayed at time = 0, 2, 4, 12 weeks.

Results: Experiments 5A and 5B revealed that following storage for 12 weeks at room temperature and 70°C, there was significant budesonide degradation when stored in acidic propylene glycol solutions. Figure 13 shows the mean (SD) percentage budesonide remaining following storage at room temperature (also shown for reference is a control experiment under

anaerobic condition, Expt 1A). Figure 14 shows the mean (SD) percentage budesonide remaining following 12 weeks of storage at 70°C (also shown for reference is a control experiment under anaerobic condition, Expt 1B). Figures 13 and 14 have error bars showing standard deviations (n=3). Figures 13 and 14 demonstrate that stress conditions of acidity accelerate budesonide degradation when stored under aerobic conditions. The ratio of the R- and S-budesonide epimers in acidic PG solution remained unchanged following storage at room temperature for 12 weeks even though the budesonide remaining in solution indicated that degradation was taking place. After 12 weeks at 70°C under acidic conditions, the R and S epimer ratio became 61.4/38.6. Thus, under acidic solution conditions, the R epimer was more stable than the S epimer.

4. Effect of Light

Methods: The following stability experiment was performed using solutions of budesonide in propylene glycol exposed to fluorescent light.

Expt. 6. 0.5%$^{w}/_{w}$ budesonide (batch number NT0038) in propylene glycol was stored at room temperature in sealed clear glass ampules placed directly under a fluorescent lamp (2750 Lux) for 4 weeks. In particular, testing for the effects of UV/VIS light followed the ICH Harmonized Tripartite Guideline, "Stability Testing: Photostability Testing of New Drug Substances and Products" as set forth November 6, 1996, by the ICH Steering Committee, which is incorporated herein by reference. Illumination of 2.3 million lux hours was achieved using a cool white fluorescent lamp. The solution and vial headspace were purged with nitrogen to produce anaerobic conditions. A control solution was protected from exposure to the light source by being enclosed in aluminum foil and similarly stored. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0).

Results: Figure 4 shows the chromatographic profile for the starting

solution prior to light exposure. Figure 19 reveals significant degradation following 4 weeks exposure to light (3.3 % = percentage budesonide remaining compared to control sample). This is clear evidence of the need to protect solutions of budesonide in propylene glycol from exposure to light.

5. Effect of water

Methods: The following stability experiments were performed using budesonide in mixtures of propylene glycol and water.

Expt. 7A. 0.5%$^{w}/_{w}$ budesonide (batch number NT0038) in a propylene glycol/water (90/10 %$^{w}/_{w}$) was prepared and stored at 40°C in a 100 ml sealed amber colored bottle. The solution and headspace were purged with oxygen each day to maintain constant environmental conditions. The solution was protected from exposure to light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0). Samples were taken and assayed at time = 0, 2, 4, 6, 8, 10, 12 weeks.

Expt. 7B. 0.05%$^{w}/_{w}$ budesonide (batch number NT0038) in propylene glycol/water (50/50%$^{w}/_{w}$) was prepared and stored at 40°C in a 100 ml sealed amber colored bottle. The solution and headspace were purged with oxygen each day in order to maintain constant environmental conditions. This solution was protected from exposure to light. Immediately following preparation, the test solution was assayed for budesonide content and epimer ratio using assay method 2 (time=0). Samples were taken and assayed at time = 0, 2, 4, 6, 8, 10, 12 weeks.

Results: Figure 20 shows that under aerobic conditions the addition of 10% water by weight to the propylene glycol vehicle improves the stability of budesonide during a 12 week stability study. (Figure 20 also shows a control experiment, using the same budesonide concentration without water.) This effect of water on budesonide stability was also shown for a solution

containing 0.05%$^{w}/_{w}$ budesonide in propylene glycol/water (50/50 by weight).

Figure 21 reveals the rate and extent of degradation of the lower budesonide concentration solution was altered in the presence of water over a 12 week study period. The mean (SD) percentage of S epimer remaining after 12 weeks storage with water present was 80.5 (1.1)% of the starting amount, compared to 58.0 (2.1)% in the absence of water. The mean (SD) percentage of R epimer remaining after 12 weeks storage with water present was 44.6. (0.9)% of the starting amount, compared to 23.7 (1.0)% in the absence of water. This suggests that budesonide formulations using the pure S epimer of budesonide will have greater stability than either the pure R epimer or the racemic mixture. While not wishing to be bound by theory, the mechanism of stabilization may in part be due to a reduced concentration of the reactant oxygen in the presence of water due to solubility changes in the vehicle.

The above described examples are indicative of the features of the invention claimed herein. While the invention and examples have been set forth using specific methods and materials, other methods and materials as known to practitioners in the art may be substituted therefor and are encompassed by this invention. The scope of the invention is set forth in the following claims.

What Is Claimed Is:

1. A stable budesonide solution comprising budesonide and a solvent, wherein after about 12 weeks, budesonide is present in the solution in an amount of at least about 90% of an initial budesonide concentration in the solution.

2. The stable budesonide solution of claim 1, wherein after about 12 weeks budesonide is present in the solution in an amount of at least about 99% of the initial budesonide concentration.

3. The stable budesonide solution of claim 1, wherein after about 28 weeks, budesonide is present in the solution in an amount of at least about 90% of the initial budesonide concentration.

4. The stable budesonide solution of claim 3, wherein after about 28 weeks budesonide is present in the solution in an amount of at least about 99% of the initial budesonide concentration.

5. The stable budesonide solution of claim 1, wherein the budesonide solution is substantially free of oxidative material.

6. The stable budesonide solution of claim 5, wherein the oxidative material is oxygen.

7. The stable budesonide solution of claim 1, wherein the initial budesonide concentration is in an amount of up to about $1.6\%^{w}/_{w}$ of the solvent.

8. The stable budesonide solution of claim 1, wherein the initial concentration of budesonide is in an amount of at least about $0.5\%^{w}/_{w}$ of the solvent.

9. The stable budesonide solution of claim 1, wherein the solvent is selected from water, a glycol or a combination thereof.

10. The stable budesonide solution of claim 9, wherein the glycol is propylene glycol or triethylene glycol.

11. The stable budesonide solution of claim 9, wherein the solvent comprises from about 10% by weight to about 50% by weight water and from about 90% by weight to about 50% by weight of the glycol.

12. The stable budesonide solution of claim 1, wherein the solution is maintained in an environment free of light.

13. The stable budesonide solution of claim 1, wherein the budesonide is S-epimer budesonide.

14. A method of stabilizing a budesonide solution comprising budesonide and a solvent, wherein the method comprises a step of:

removing oxidative material from the budesonide solution.

15. The method of claim 14, wherein the oxidative material is oxygen.

16. The method of claim 14, wherein the step of removing oxidative material comprises sparging the budesonide solution with a non-reactive gas.

17. The method of claim 16, wherein the non-reactive gas is nitrogen.

18. The method of claim 16, further comprising a step of storing the sparged budesonide solution in a container.

19. The method of claim 18, wherein the step of storing the sparged budesonide solution further comprises:

purging the container with the non-reactive gas; and

filling the purged container with the sparged budesonide solution.

20. The method of claim 19, further comprising a step of sealing the filled container such that a headspace in the container is free of oxygen.

21. The method of claim 19, further comprising a step of purging a headspace of the filled container with the non-reactive gas before sealing the container.

22. The method of claim 18, wherein the step of storing the sparged budesonide solution further comprises maintaining the budesonide solution in an environment that is free of light, free or air, or free of both light and air.

23. The method of claim 18, wherein the step of storing the sparged budesonide solution further comprises maintaining the budesonide solution at about room temperature.

24. The method of claim 14, further comprising a step of preparing the budesonide solution by mixing budesonide with the solvent selected from water, a glycol or a combination thereof.

25. The method of claim 24, wherein the glycol is propylene glycol or triethylene glycol.

26. The method of claim 24, wherein the solvent comprises from about 10% by weight to about 50% by weight water and from about 90% by weight to about 50% by weight glycol.

27. The method of claim 24, wherein the budesonide solution is prepared with budesonide in an amount of at least about 0.5% $^{w}/_{w}$ of the solvent.

28. The method of claim 24, further comprising selecting budesonide to be S-epimer budesonide.

29. A method of storing budesonide, comprising:

preparing a budesonide solution comprising budesonide and a solvent;

sparging the budesonide solution with a non-reactive gas to remove oxygen;

purging a storage container with a non-reactive gas to remove oxygen;

filling the purged storage container with the sparged budesonide solution;

purging a headspace of the filled storage container with a non-reactive gas to remove oxygen; and

sealing the container.

30. The method of claim 29, further comprising maintaining the budesonide solution in an environment that is free of light, free or air, or free of both light and air.

31. The method of claim 29, further comprising maintaining the budesonide solution at about room temperature.

32. The method of claim 29, wherein the solvent is selected from water, a glycol or a combination thereof.

33. The method of claim 32, wherein the glycol is propylene glycol or triethylene glycol.

34. The method of claim 32, wherein the solvent comprises from about 10% by weight to about 50% by weight water and from about 90% by weight to about 50% by weight glycol.

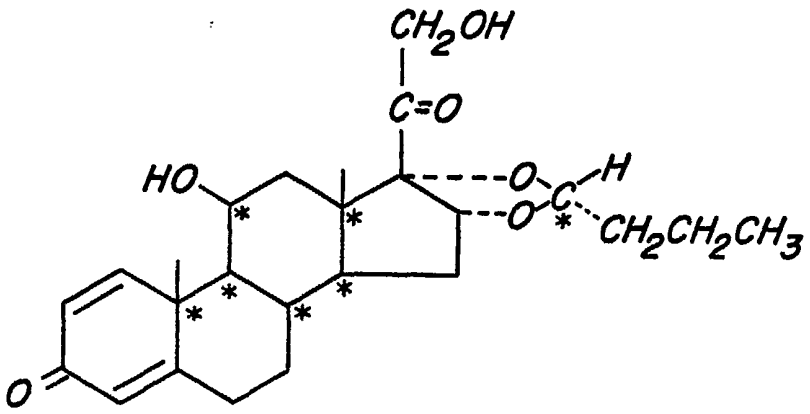
35. The method of claim 29, wherein the budesonide solution is prepared with budesonide in an amount of at least about 0.5%$^{w}/_{w}$ of the solvent.

36. A stable budesonide solution prepared by the method of claim 29, wherein after about 12 weeks, budesonide is present in the solution in an amount of at least about 90% of an initial budesonide concentration.

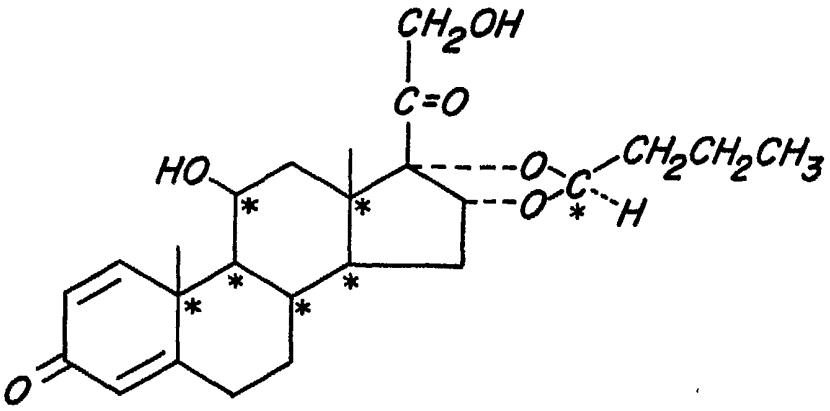
37. The stable budesonide solution of claim 36, wherein after about 12 weeks budesonide is present in the solution in an amount of at least about 99% of the initial budesonide concentration.

38. The stable budesonide solution of claim 36, wherein after about 28 weeks, budesonide is present in the solution in an amount of at least about 90% of the initial budesonide concentration.

39. The stable budesonide solution of claim 36, wherein after about 28 weeks budesonide is present in the solution in an amount of at least about 99% of the initial budesonide concentration.



Epimer 22R of budesonide



Epimer 22S of budesonide

* indicate chiral centers

Fig. 1

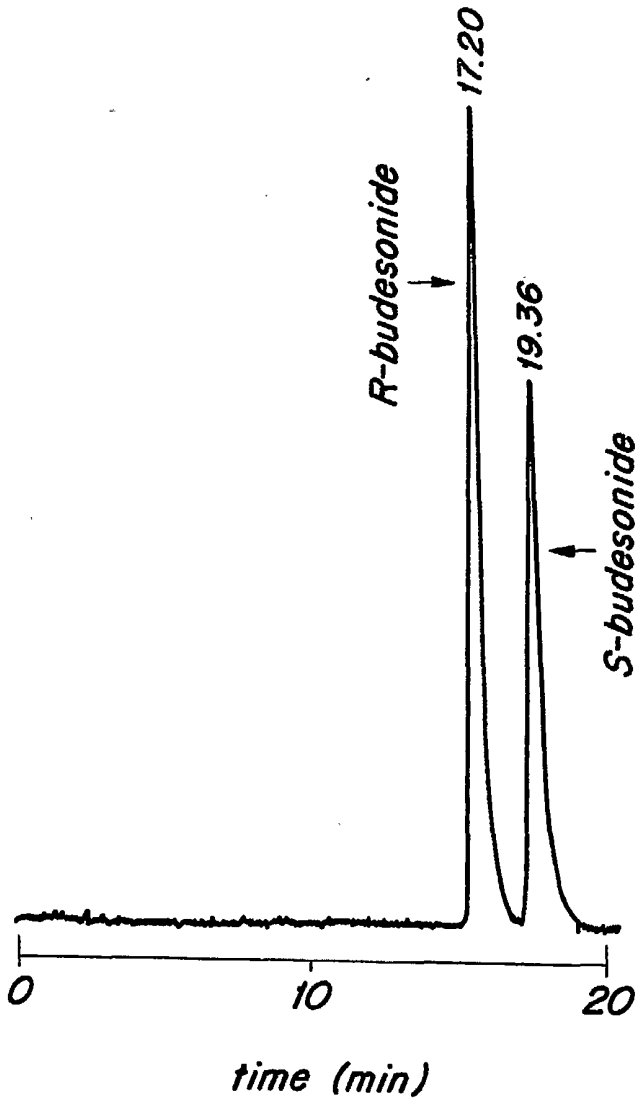


Fig. 2

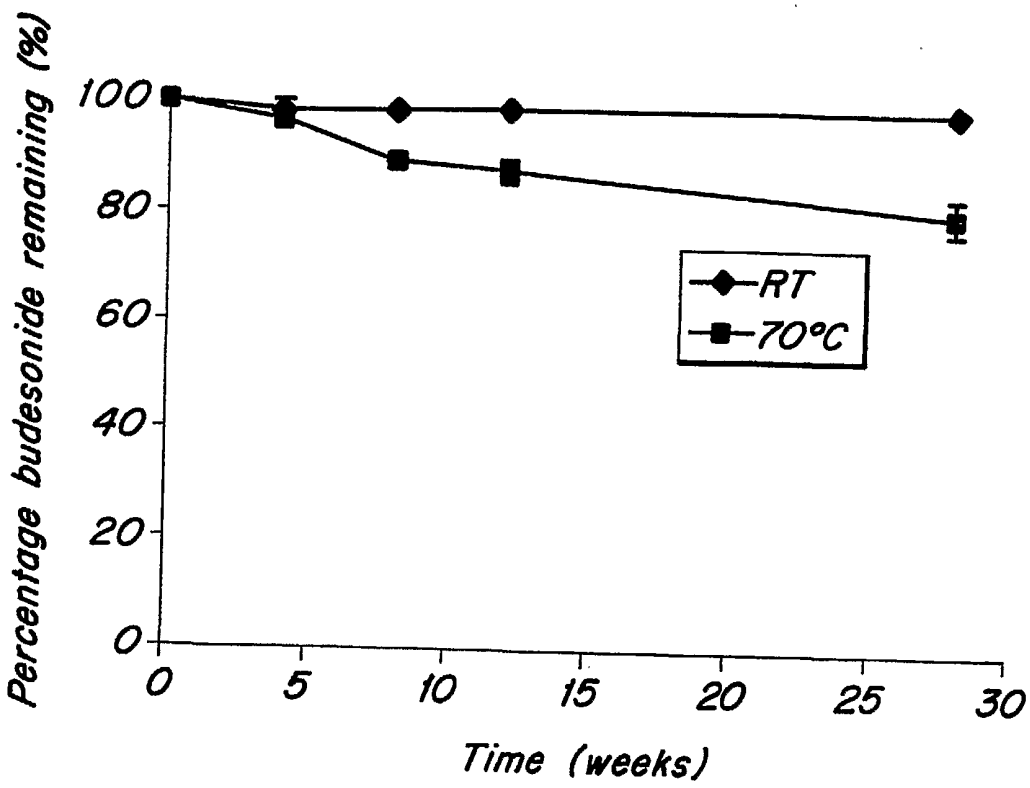

Percentage budesonide remaining (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (weeks)
RT
70°C

Fig. 3

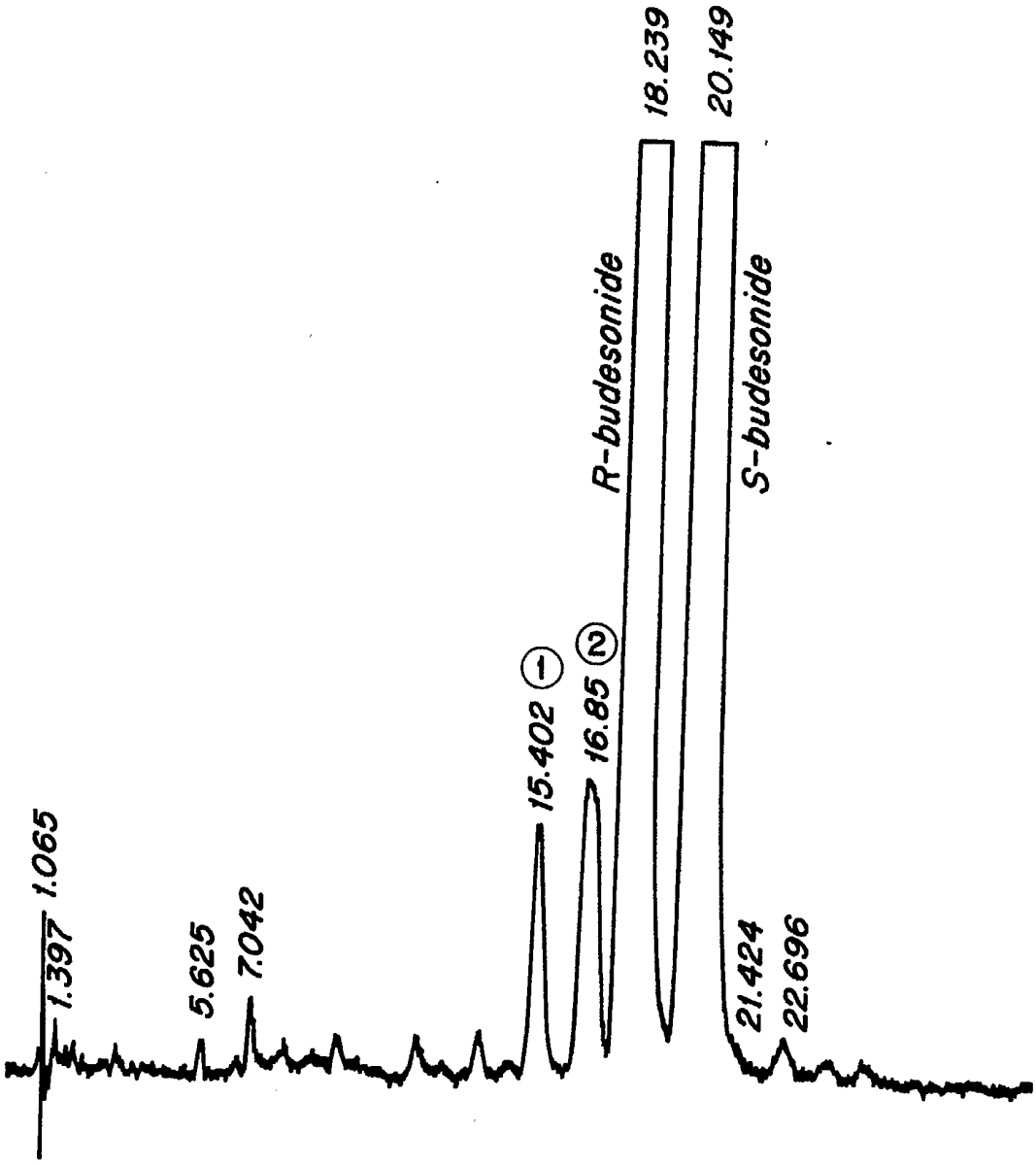

1.065
1.397
5.625
7.042
15.402
16.85
R-budesonide
18.239
20.149
S-budesonide
21.424
22.696

Fig. 4

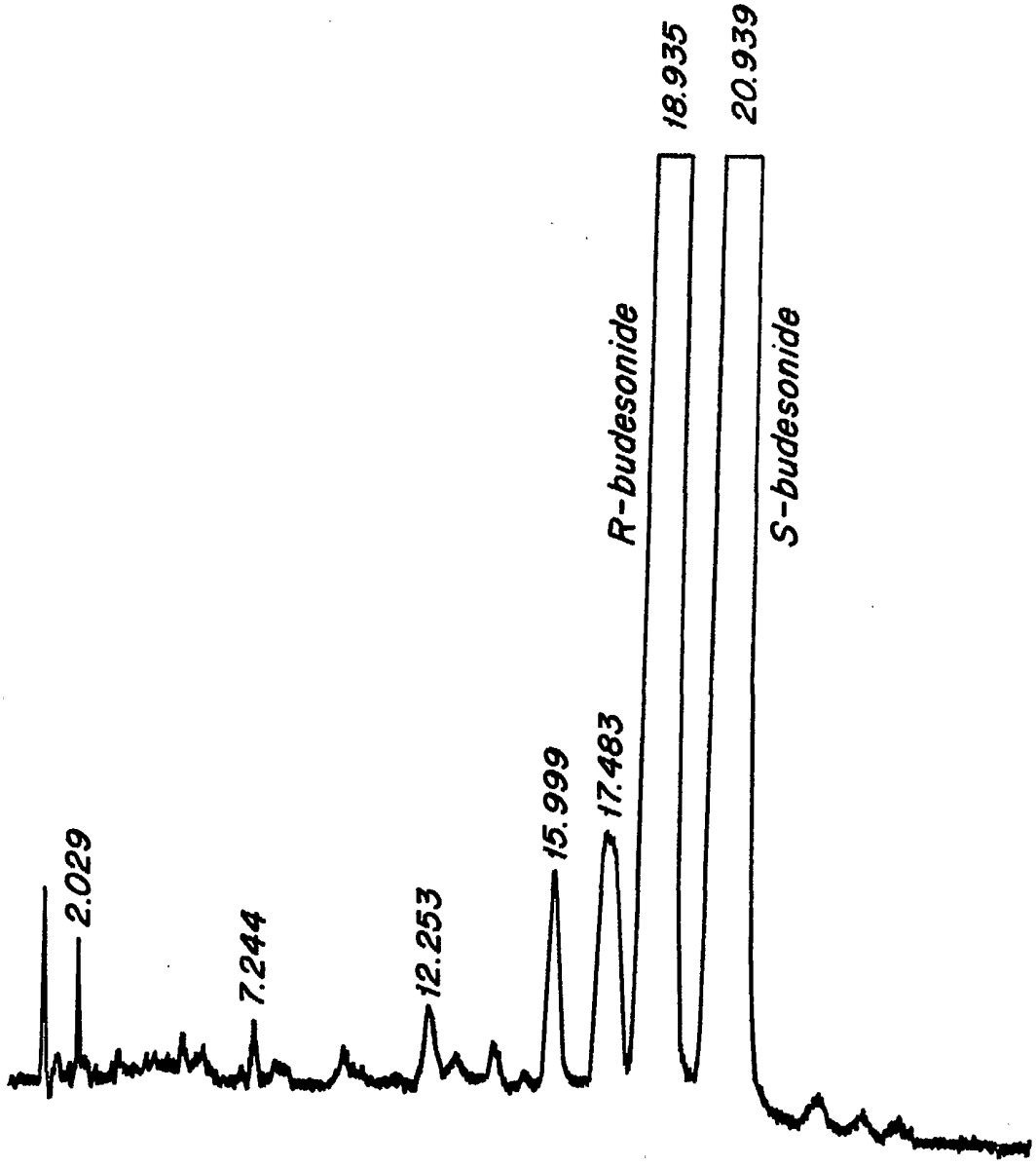
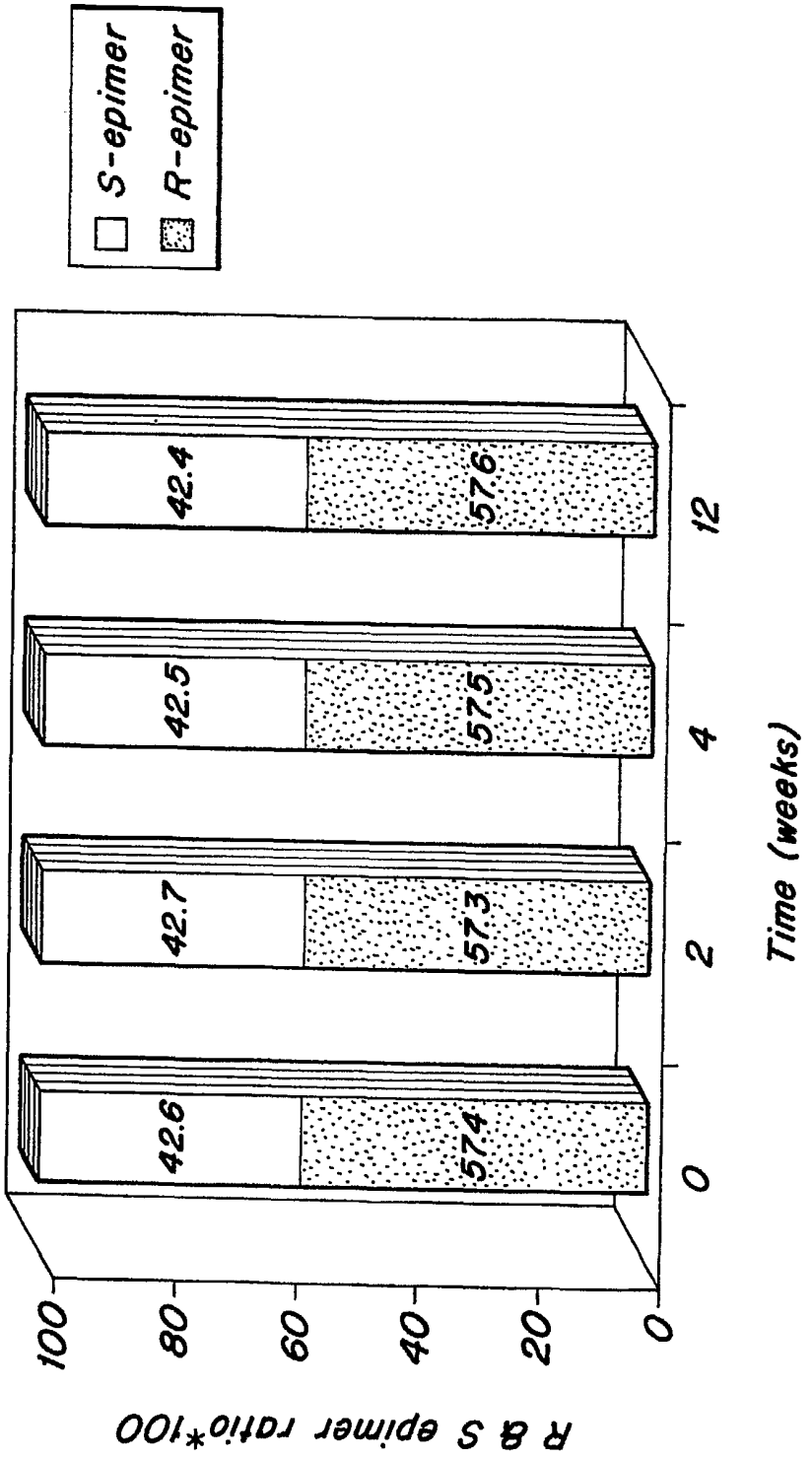

2.029
7.244
12.253
15.999
17.483
R-budesonide
18.935
20.939
S-budesonide

Fig. 5

Fig. 6


S-epimer
R-epimer
42.4
57.6
42.5
57.5
42.7
57.3
42.6
57.4
0
2
4
12
Time (weeks)
100
80
60
40
20
0
R & S epimer ratio*100

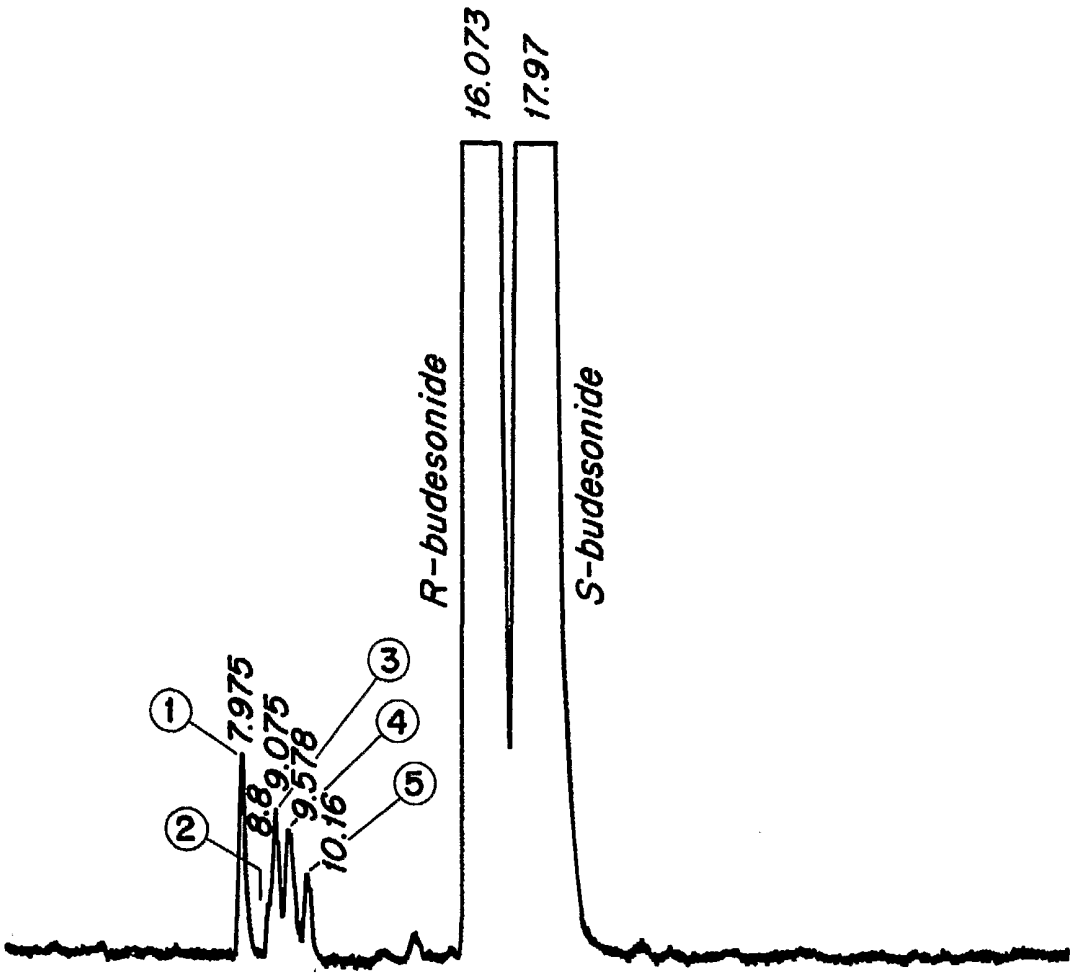

16.073
17.97
R-budesonide
S-budesonide
1
2
3
4
5
7.975
8.8
9.075
9.578
10.16

Fig. 7

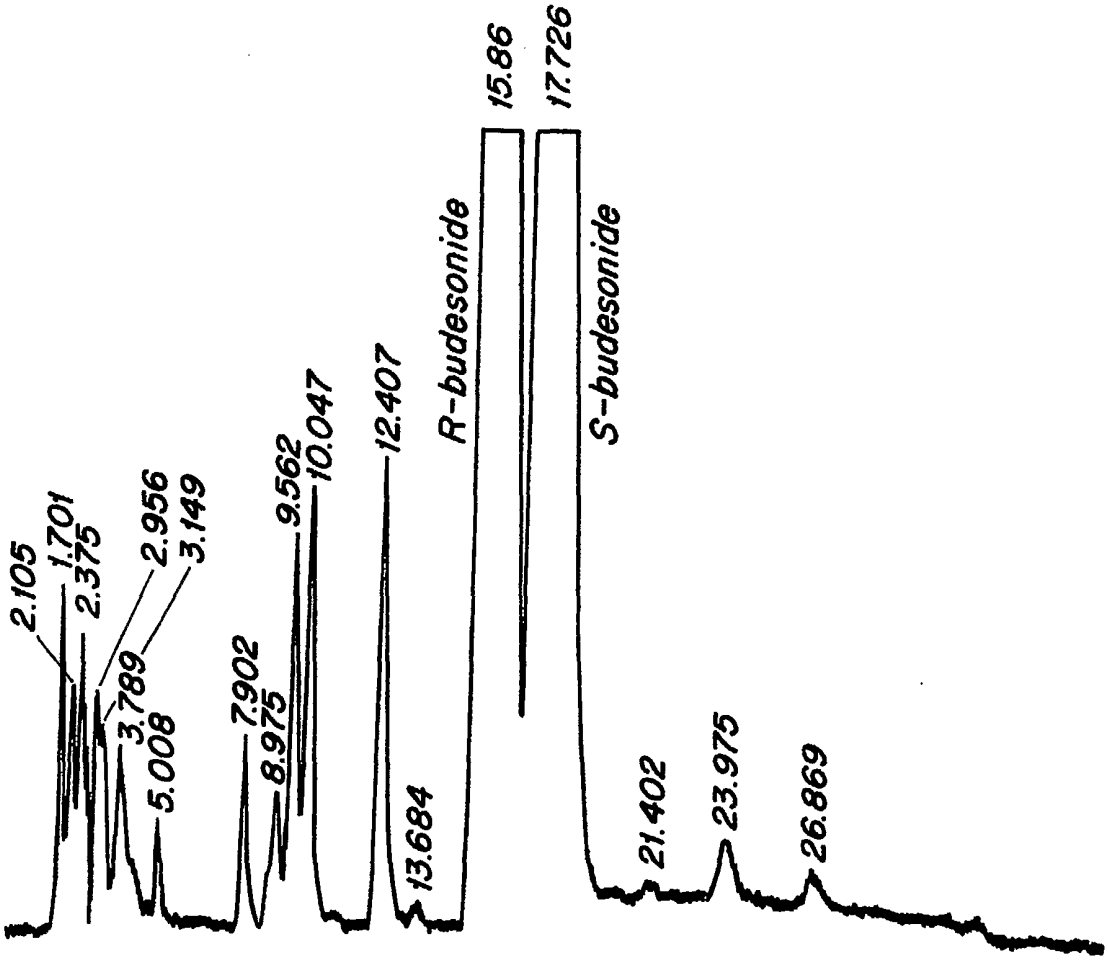

2.105
1.701
2.375
2.956
3.149
3.789
5.008
7.902
8.975
9.562
10.047
12.407
13.684
15.86
17.726
R-budesonide
S-budesonide
21.402
23.975
26.869

Fig. 8

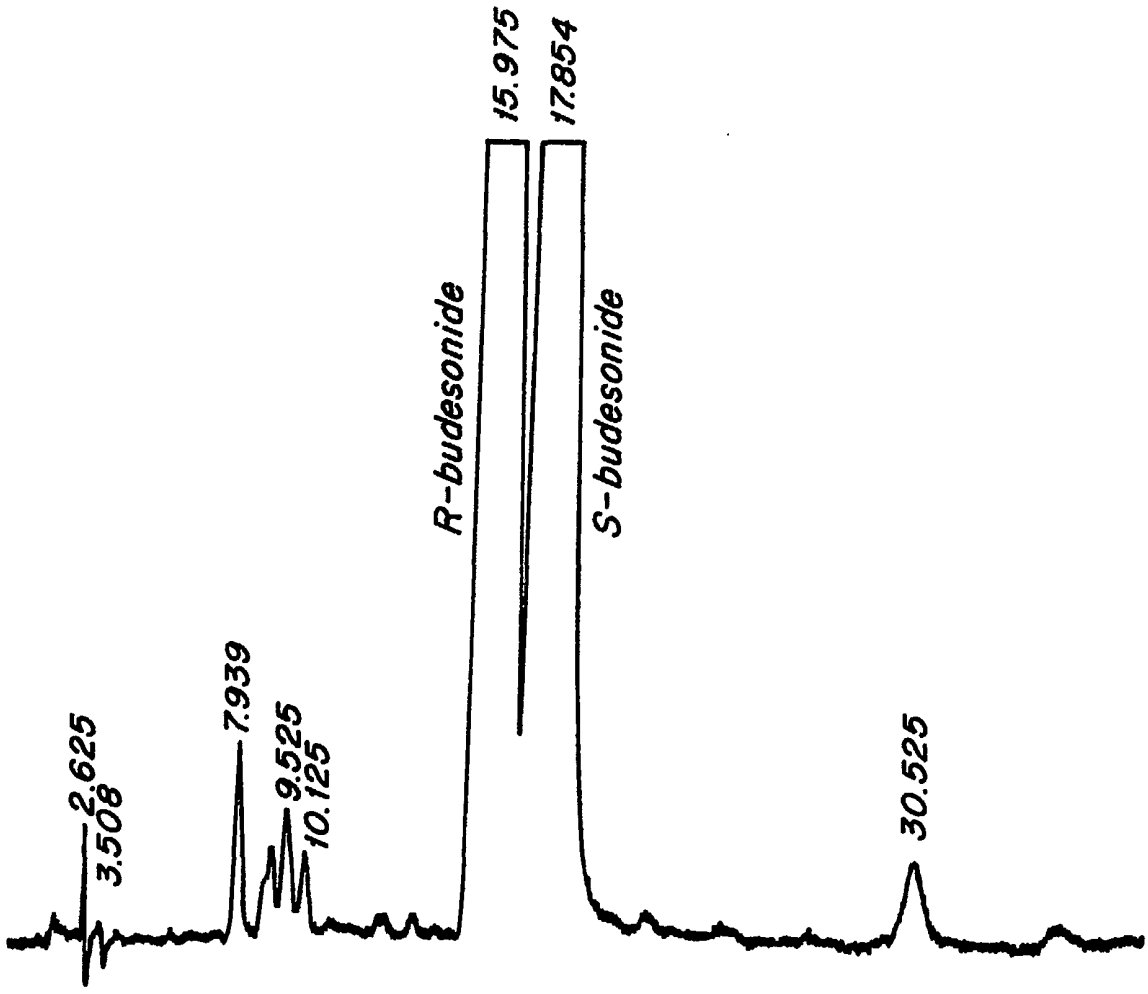
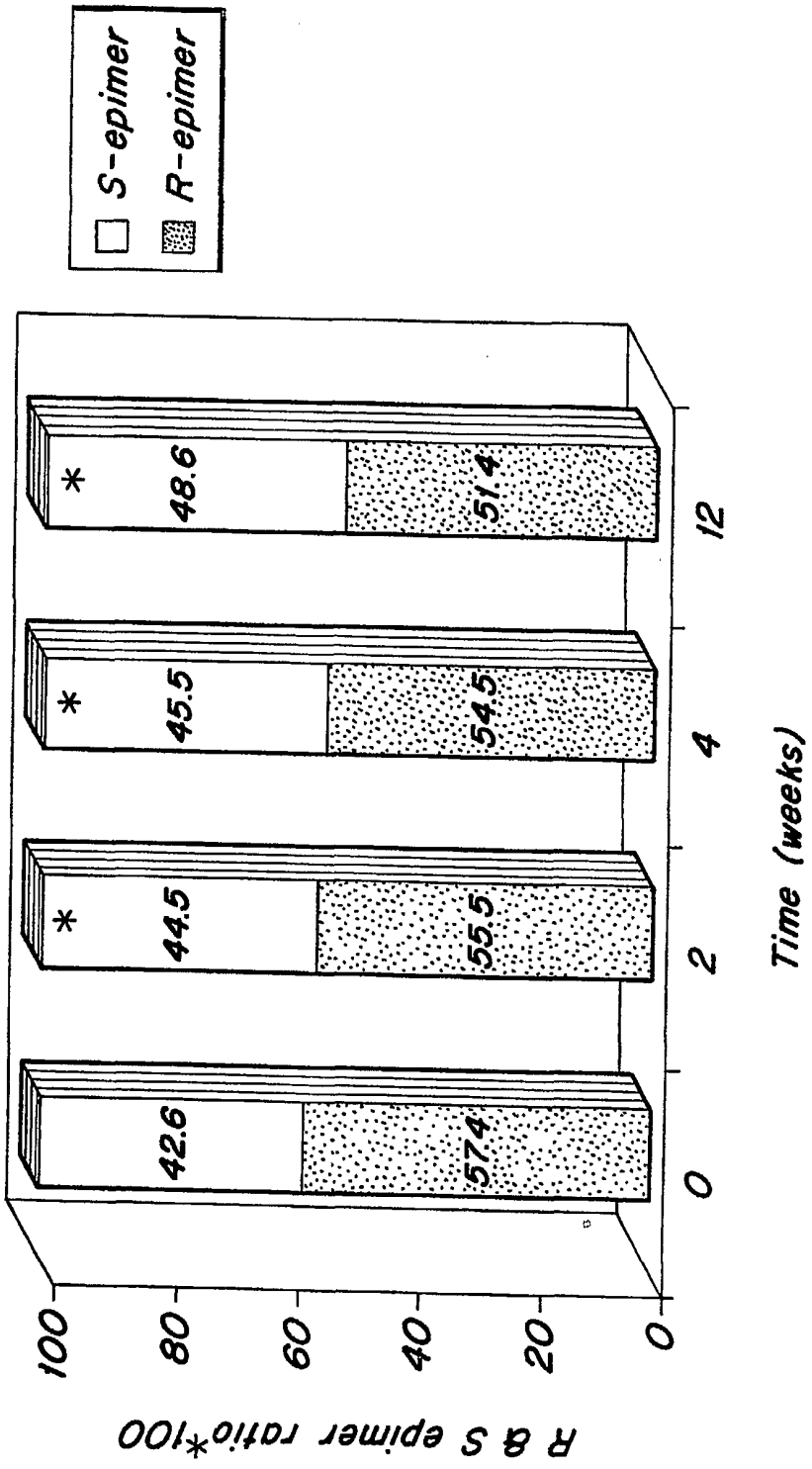

2.625
3.508
7.939
9.525
10.125
R-budesonide
15.975
17.854
S-budesonide
30.525

Fig. 9

Fig 10


S-epimer
R-epimer
48.6
51.4
45.5
54.5
44.5
55.5
42.6
57.4
*
0
2
4
12
Time (weeks)
0
20
40
60
80
100
R & S epimer ratio*100

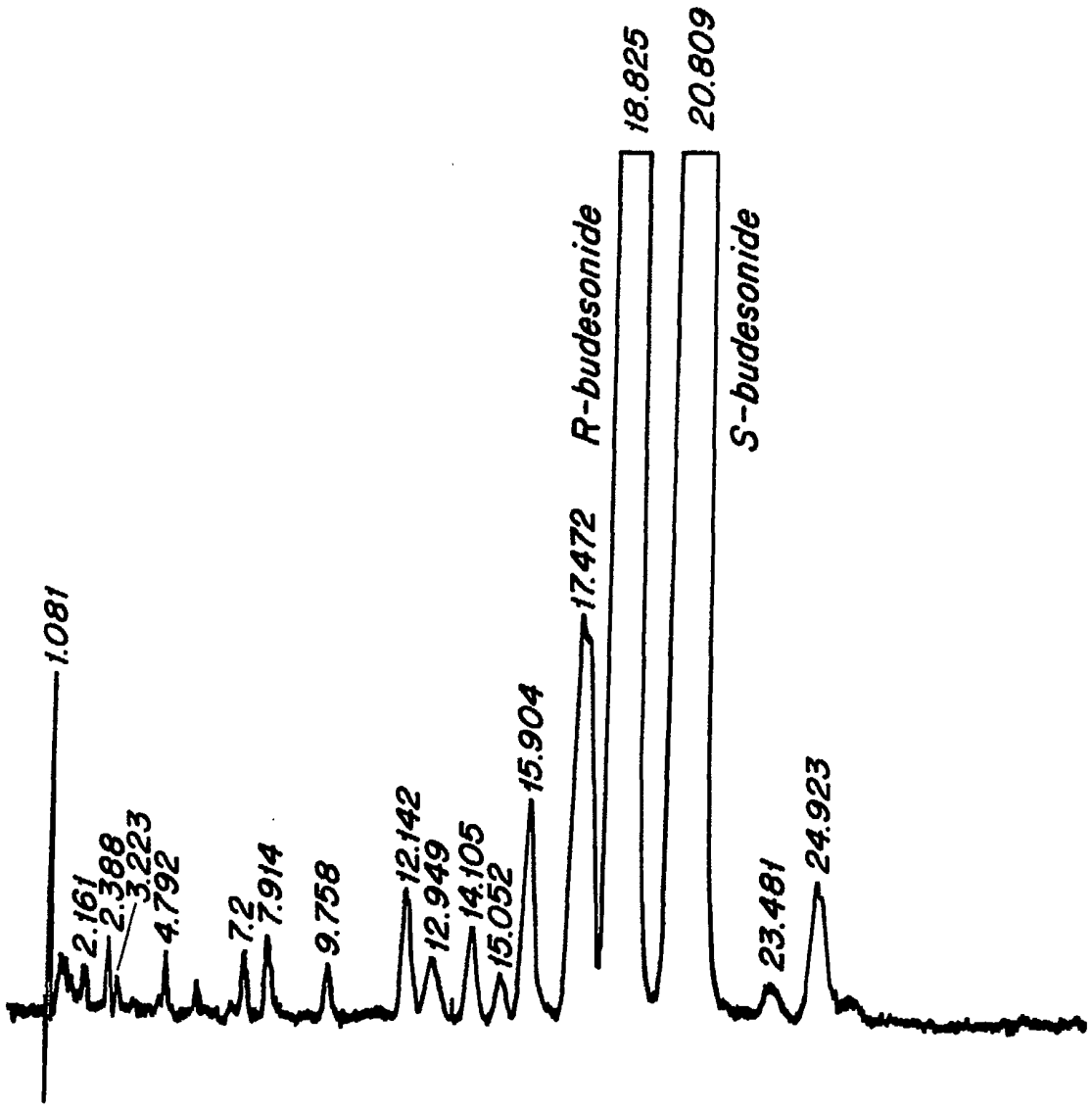

1.081
2.161
2.388
3.223
4.792
7.2
7.914
9.758
12.142
12.949
14.105
15.052
15.904
17.472
R-budesonide
18.825
20.809
S-budesonide
23.481
24.923

Fig. 11

Fig. 12

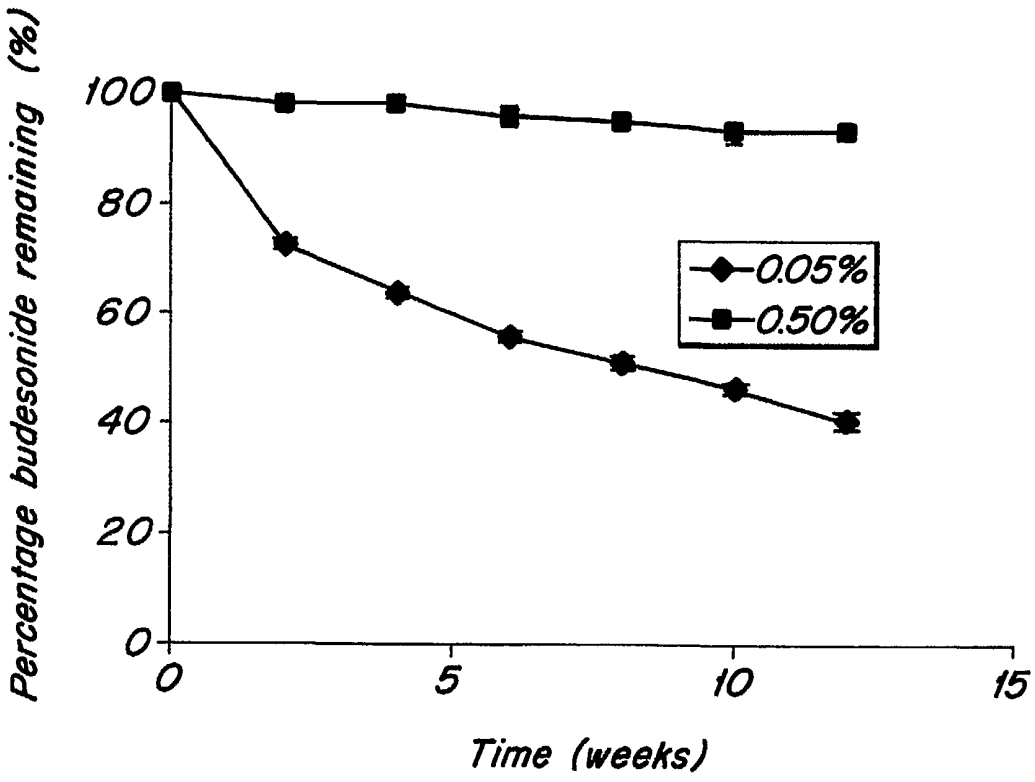

Percentage budesonide remaining (%)
100
80
60
40
20
0
0
5
10
15
Time (weeks)
0.05%
0.50%

Fig. 13

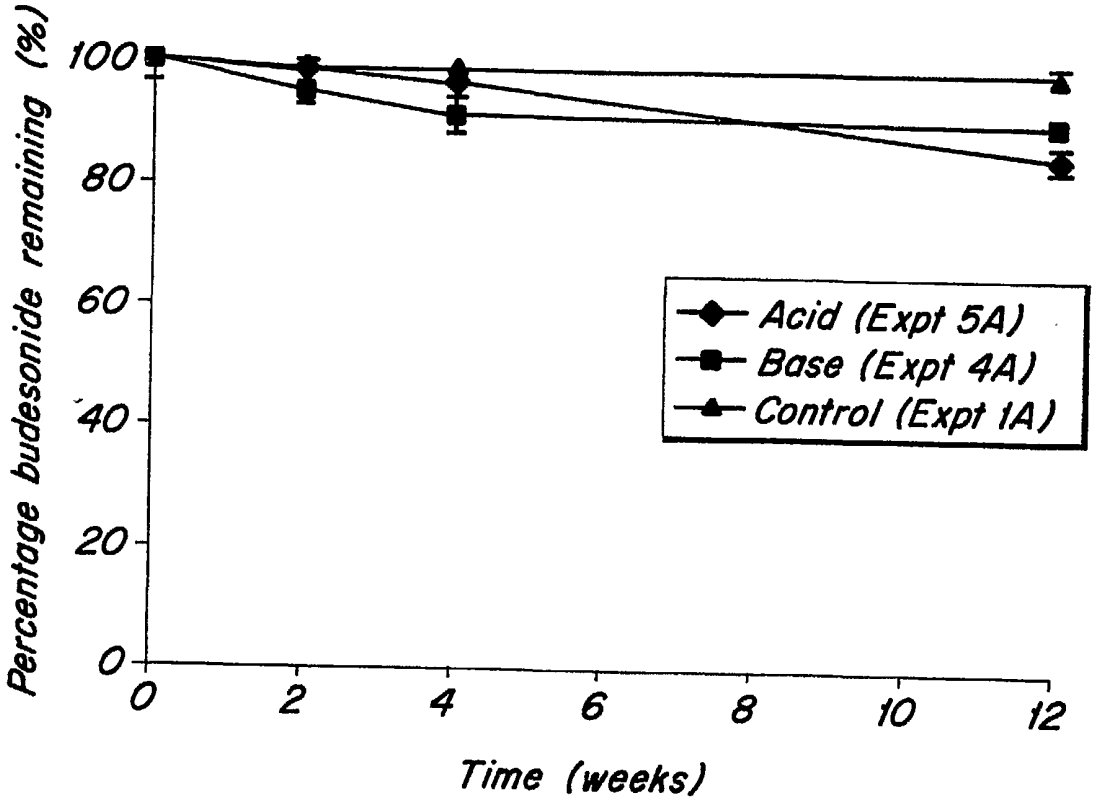

Percentage budesonide remaining (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
Time (weeks)
Acid (Expt 5A)
Base (Expt 4A)
Control (Expt 1A)

Fig. 14

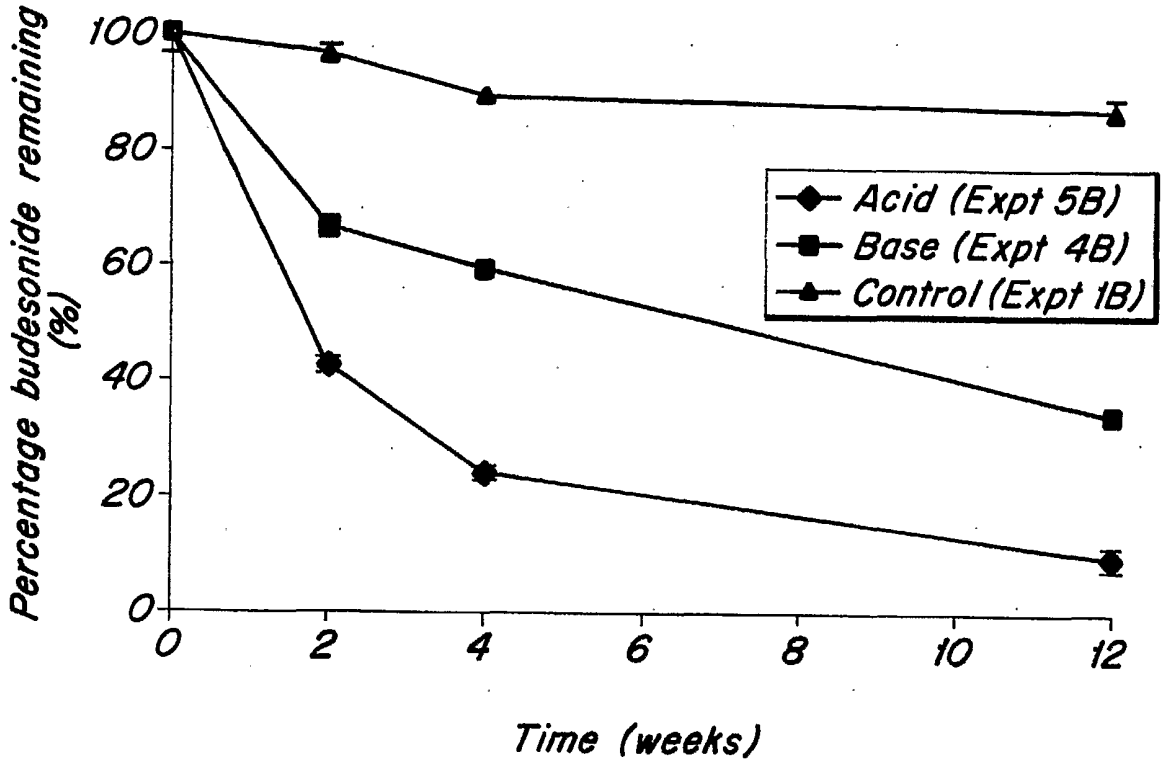
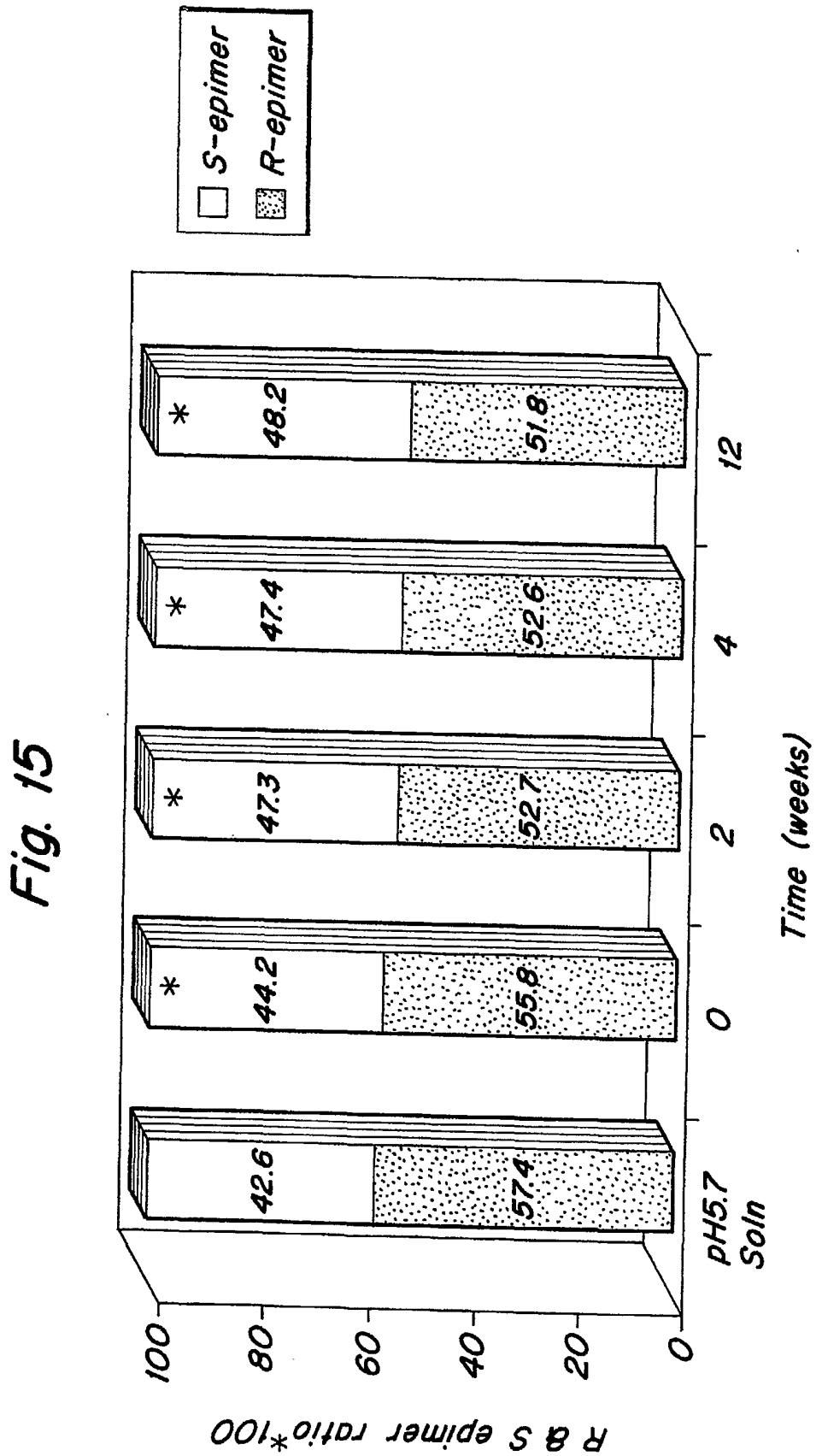

Percentage budesonide remaining (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
Time (weeks)
Acid (Expt 5B)
Base (Expt 4B)
Control (Expt 1B)

Fig. 15


S-epimer
R-epimer
48.2
51.8
47.4
52.6
47.3
52.7
44.2
55.8
42.6
57.4
*
pH5.7 Soln
0
2
4
12
Time (weeks)
R & S epimer ratio*100
0
20
40
60
80
100

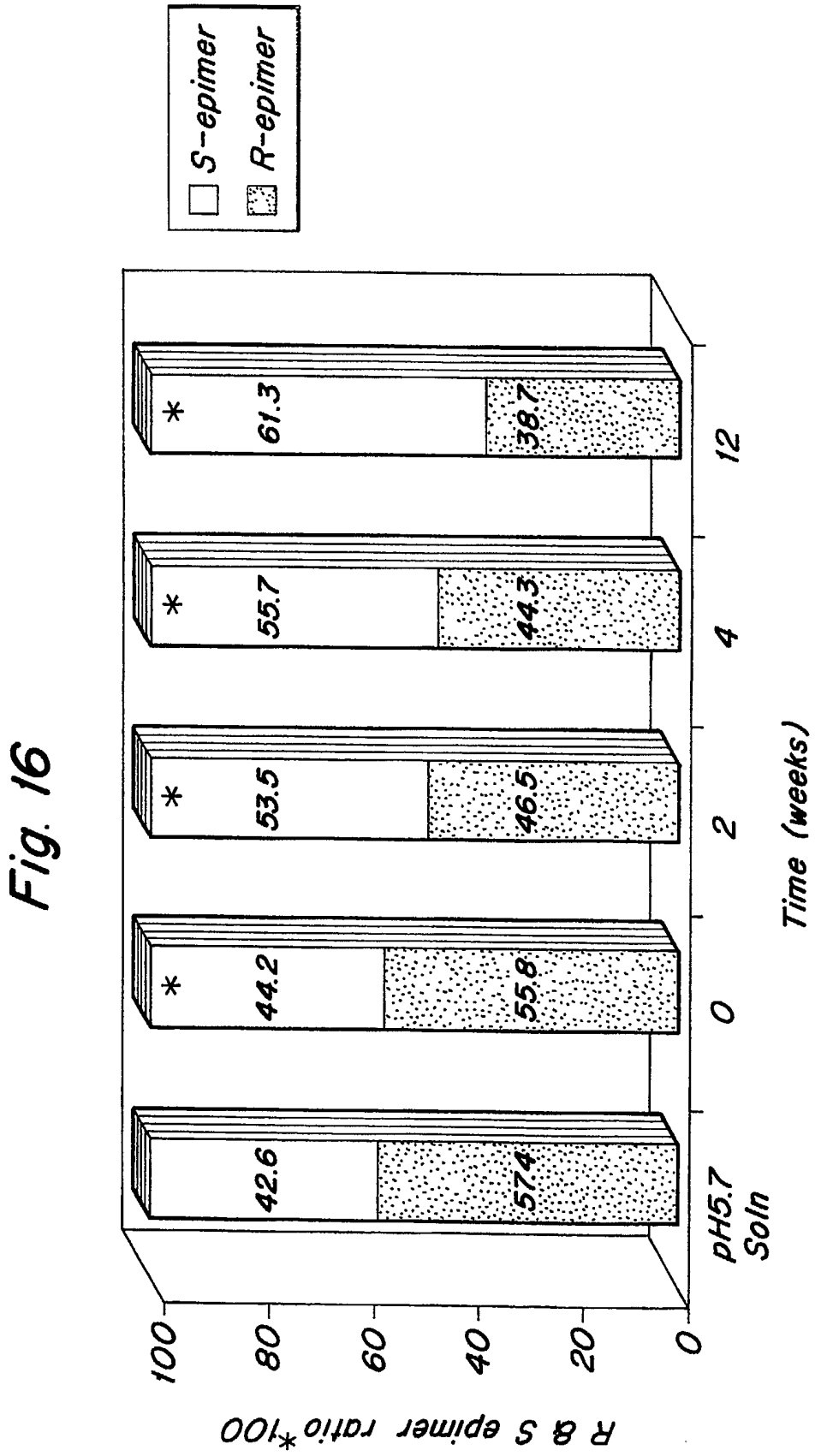

S-epimer
R-epimer
*
61.3
38.7
55.7
44.3
53.5
46.5
44.2
55.8
42.6
57.4
12
4
2
0
Time (weeks)
pH5.7 Soln
100
80
60
40
20
0
R & S epimer ratio *100

Fig. 16

Fig. 17

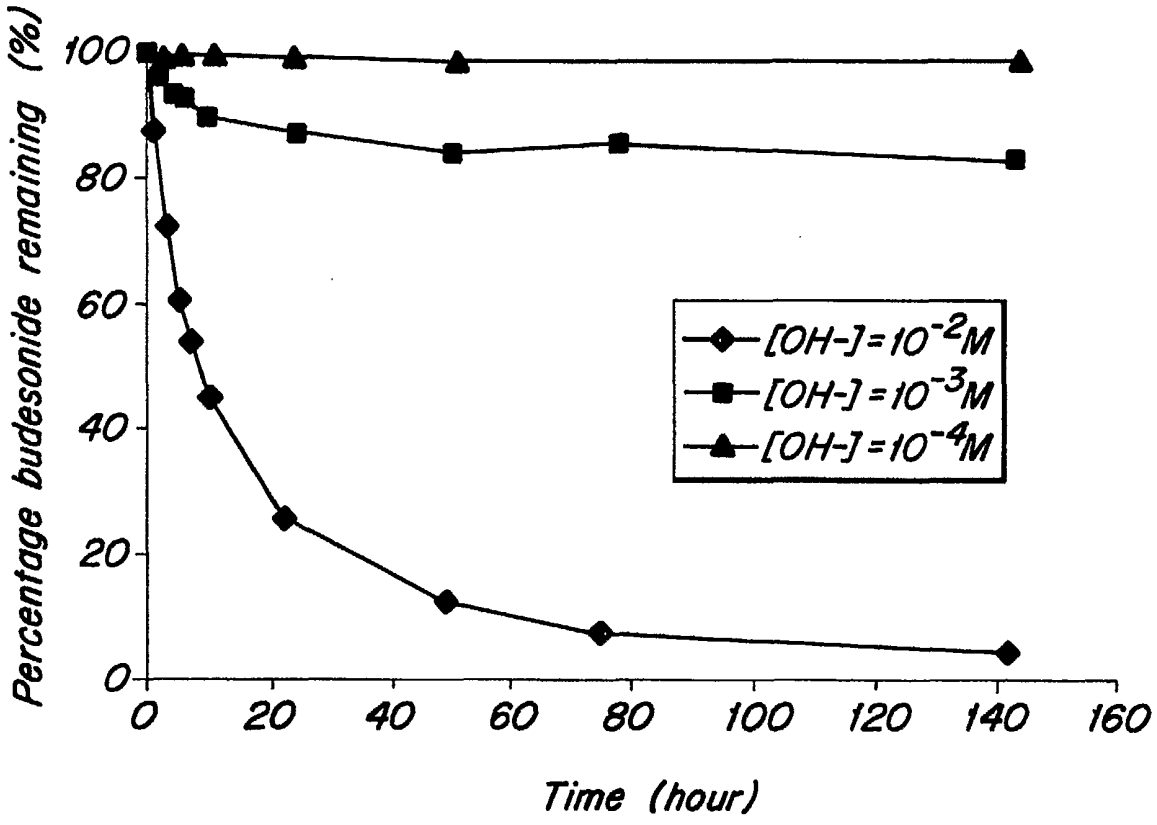
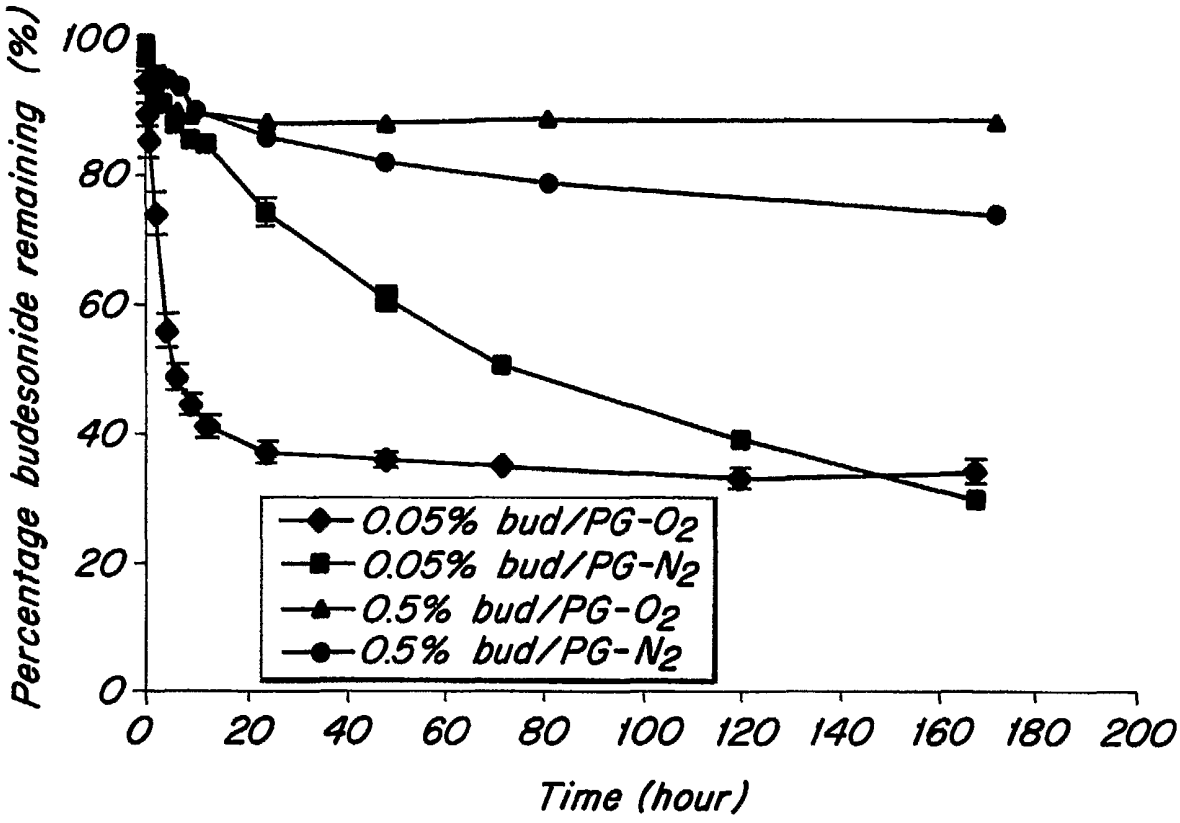

Percentage budesonide remaining (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
Time (hour)
[OH-] = 10-2M
[OH-] = 10-3M
[OH-] = 10-4M

Fig. 18


Percentage budesonide remaining (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
200
Time (hour)
0.05% bud/PG-O2
0.05% bud/PG-N2
0.5% bud/PG-O2
0.5% bud/PG-N2

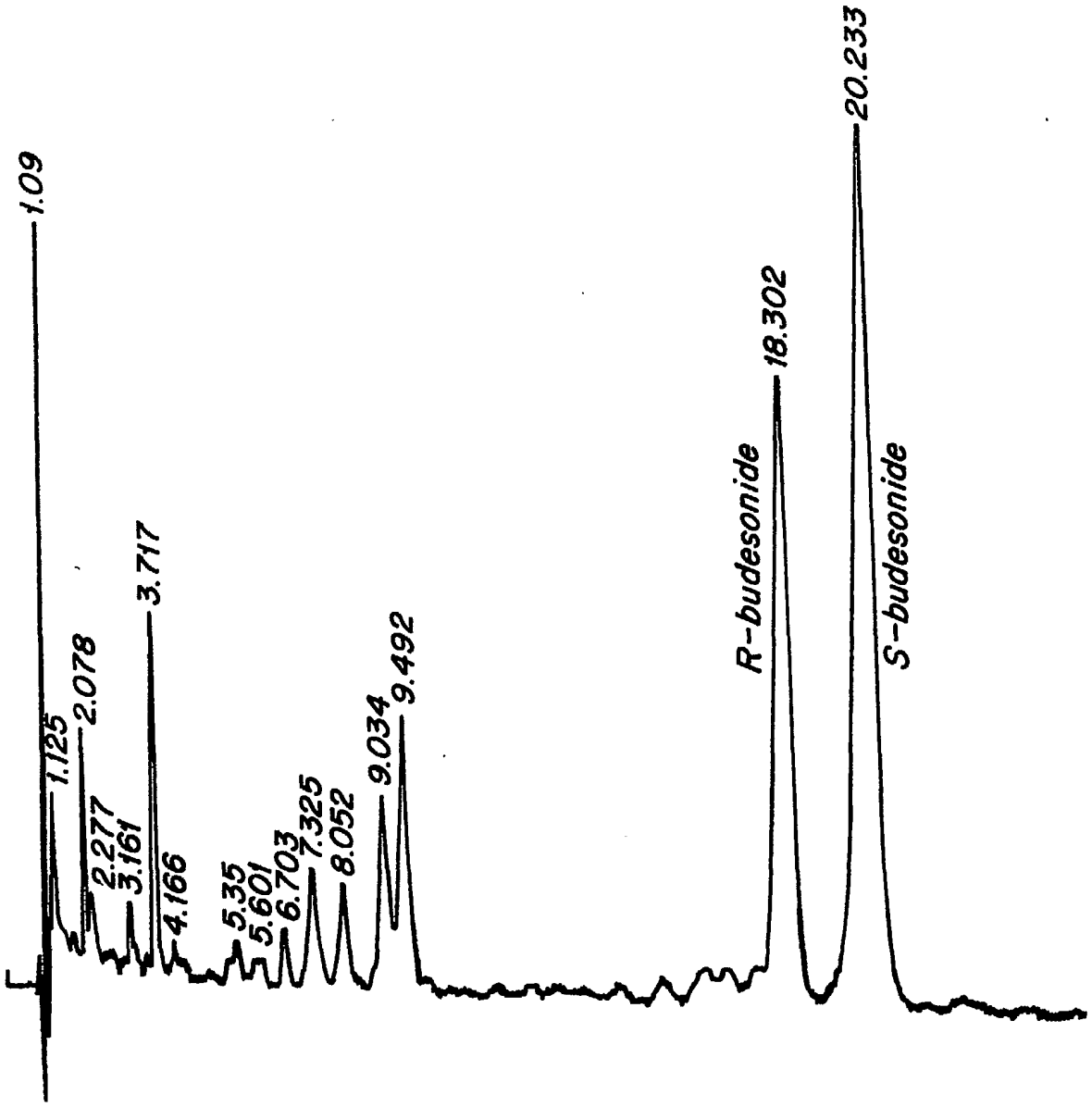

1.09
1.125
2.078
2.277
3.161
3.717
4.166
5.35
5.601
6.703
7.325
8.052
9.034
9.492
R-budesonide
18.302
20.233
S-budesonide

Fig. 19

Fig. 20

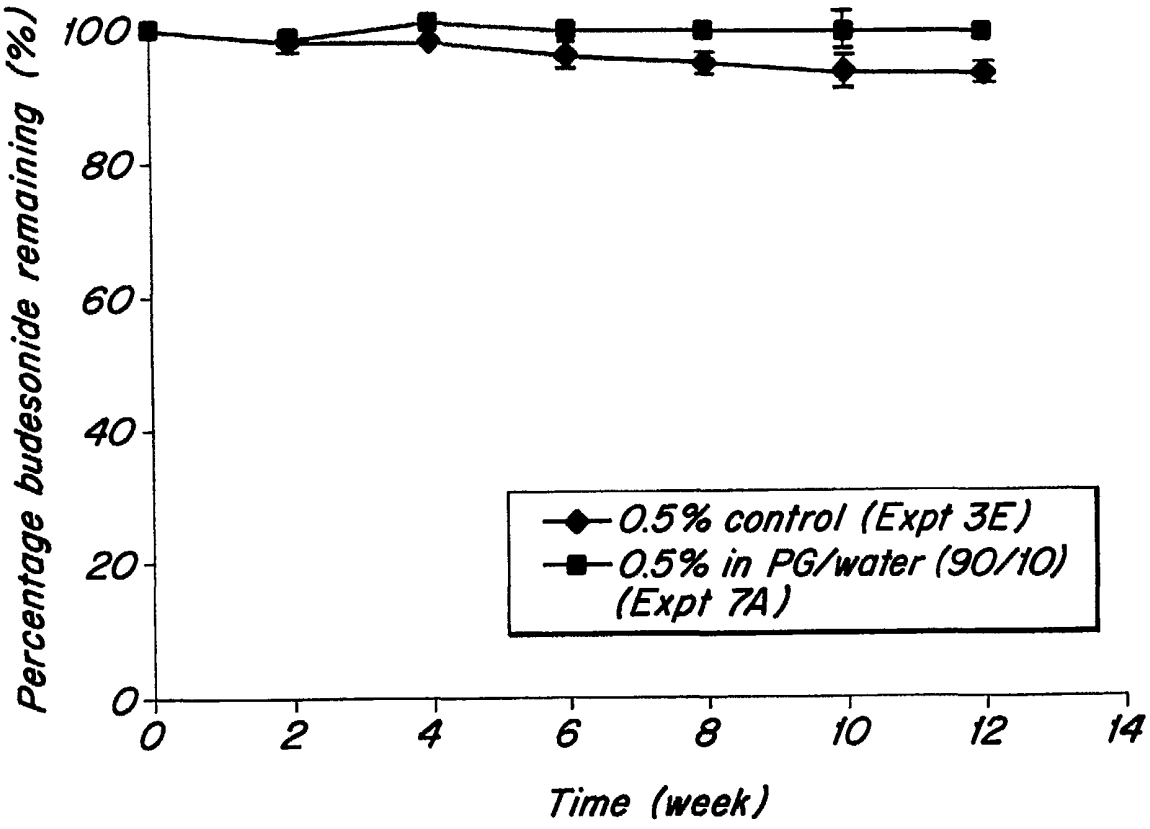
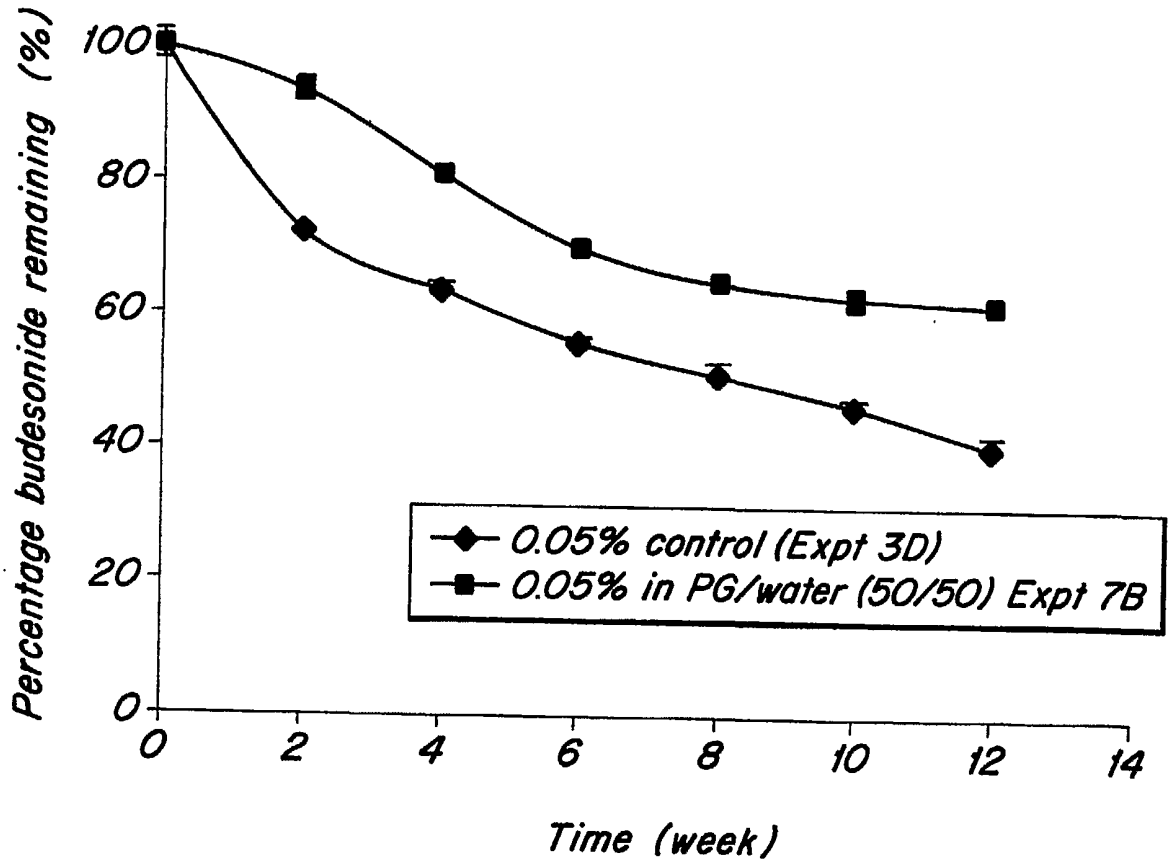

Percentage budesonide remaining (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time (week)
0.5% control (Expt 3E)
0.5% in PG/water (90/10) (Expt 7A)

Fig. 21


Percentage budesonide remaining (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time (week)
0.05% control (Expt 3D)
0.05% in PG/water (50/50) Expt 7B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/40581

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61F 13/00, 13/02; A61K 9/48

US CL : 424/431, 433, 434, 435, 451

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)

U.S. : 424/431, 433, 434, 435, 451

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 practicable, search terms used)

West and NPL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,914,122 A (OTTERBECK et al.) 22 June 1999 (22.06.1999); see column 3, lines 5-51, table 2 on column 2, column 4, lines 4-52.	1-3, 5-13 --- 4, 39
Y,P	US 6,076,522 A (DWIVEDI et al.) 20 June 2000 (20.06.2000); see column 4, lines 41-52, column 5, lines 45 through column 6, lines 1-24, column 7, lines 29-39.	14-38

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2001 (30.06.2001)	13 JUL 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Susan Tran Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)