

**PHARMACEUTICAL COMPOSITION CONTAINING GESTAGENS AND/OR
ESTROGENS AND 5-METHYL-(6S)-TETRAHYDROFOLATE**

The present invention relates to a pharmaceutical
5 composition which comprises progestogens, estrogens and
5-methyl-(6S)-tetrahydrofolate, can be employed as oral
contraceptive and moreover prevents disorders and
malformations caused by folate deficiency, without at
the same time masking the symptoms of vitamin B₁₂
10 deficiency.

Drugs companies active in the area of fertility control
are always making efforts to improve the available
contraceptives. Included therein is not only increasing
15 the contraceptive reliability by developing novel
substances and an improved convenience of use. On the
contrary, innovative approaches to combining
contraception and disease prevention are also being
pursued.

20 A number of diseases is regarded as being connected
with a folate deficiency. Thus, administration of
folates for example in the form of folic acid may
minimize the risk of cardiovascular disorders and
25 certain malignant disorders (such as, for example,
carcinoma of the breast or colon).

Defects in the development of unborn children are
particularly serious consequences of folate deficiency
30 in women of childbearing age. Thus, women with low
folate levels have an increased risk, compared with
those having sufficiently high folate levels, of giving
birth to children suffering from congenital
malformations such as neural tube, ventricular valve
35 and urogenital defects.

Neural tube defects are the commonest congenital
malformations of the central nervous system. They arise

through incomplete closure of the neural tube in about the third to fourth week of embryonic development. Neural tube defects include spina bifida (in some cases with meningocele or meningomyelocele), encephalocele and anencephalies which are characterized by partial or complete absence of areas of the brain. Children with anencephaly are virtually incapable of survival.

Spina bifida is distinguished by incomplete closure of vertebral arches. Its result, depending on the nature of the lesion, is life-long disability in the form of various sensory but also motor deficits - thus, for example, two thirds of children and adults are dependent on wheelchairs owing to muscular paralyses. Therapy entails covering the defect, fitting a shunt to drain the CSF and lengthy orthopedic and neurological rehabilitation. The costs of the medical treatment average 500 000 € per child.

It is assumed that there are about 250 000 neonates with neural tube defects around the world. The rate of neonatal impairments in Germany and the USA is about 1-2 per 1000 births. In Germany each year about 500 babies are born alive with neural tube defects, while a further 500 pregnancies have been terminated on the basis of prenatal ultrasonic diagnosis.

Sufficiently high folate levels at the time of conception and in the initial phase of pregnancy are crucial for avoiding neural tube defects. An erythrocyte folate level of at least 906 nmol/l is generally regarded as desirable for reducing the frequency of neural tube defects.

It is known that intake of folic acid at the right time around conception can reduce neural tube defects by 50-70%. The folic acid fortification of food products which is practiced in the USA has already markedly reduced the incidence of neural tube defects; in Canada

and Chile in fact by more than 50%.

Both voluntary fortification of food products as, for example, in Germany, and intake of folic acid products
5 does not, however, reach all women of childbearing age to a sufficient extent. Firstly, many women are unaware of the risk of neural tube defects and the possibility of minimizing a corresponding risk by intake of folic acid. Thus, in many countries, far fewer than 10% of
10 them take folic acid products around the time of conception. Secondly, despite modern methods of contraception, which are increasingly easy to use, a large number of pregnancies - estimated at up to 50% in the USA (Inst. of Medicine 1998, NEJM 2004) - are
15 unplanned, so that deliberate intake of folic acid products before conception is likewise precluded from the outset. In addition, for example in the USA, about 5-8% of users do not take oral contraceptives reliably.

20 The object on which the patent US 6,190,693 (Kafrissen et al.) was based was therefore to prevent certain disorders which can be treated by folic acid in consumers of oral contraceptives. Kafrissen achieved this object by adding folic acid to an oral
25 contraceptive. He disclosed a method for administering folic acid by use of a pharmaceutical composition which comprised both conventional substances with contraceptive activity and folic acid.

30 However, introduction of folic acid into oral contraceptives itself involves a serious health risk, because it may mask the early symptoms, which are still treatable, of a vitamin B₁₂ deficiency such as, for example, a megaloblastic anemia. This is because the
35 hematological symptoms caused by vitamin B₁₂ deficiency can be treated so well by additional folate administration that a vitamin B₁₂ deficiency can be detected only with great difficulty, or not at all, and consequently therefore not diagnosed. The

neuropsychiatric symptoms such as, for example, paresthesia and ataxia then remain untreated, however, and might deteriorate irreversibly.

5 The object on which the patent application WO 03/070255 (Coelingh Bennink) was based was therefore to avoid a health risk arising from the masking of the symptoms of a vitamin B₁₂ deficiency in consumers of folic acid-containing oral contraceptives. Coelingh Bennink
10 achieves this object by adding vitamin B₁₂ to an oral contraceptive. He discloses a kit for oral hormonal contraception which comprises estrogens and/or progestogens, tetrahydrofolates and, obligatorily, vitamin B₁₂.

15

A further problem associated with the administration of folic acid and tetrahydrofolate products - which comprise no 5-methyl-(6S)-tetrahydrofolate - is the polymorphism of methylenetetrahydrofolate reductase
20 (MTHFR C677T), which is heterozygous in about 55% of the Caucasian population and homozygous in about 10-15%. This polymorphism leads to a reduced activity of methylenetetrahydrofolate reductase, so that the women affected are unable to metabolize sufficiently
(25 the supplied folate and tetrahydrofolate into 5-methyl-(6S)-tetrahydrofolate, which is active in the body. This polymorphism is an acknowledged risk factor for disorders caused by folate deficiency, in particular for neural tube defects.

30

A further problem causing difficulties is that folic acid is a substance which does not naturally occur in foodstuffs. In order to be biologically active, it must first be converted metabolically by the enzyme
35 dihydrofolate reductase into 7,8-dihydrofolate and (6S)-tetrahydrofolate. The metabolic capacity, in particular the first activation step, for conversion of the provitamin folic acid into its active reduced form is limited and moreover varies greatly from individual

to individual. Since the enzyme dihydrofolate reductase does not play a role in the metabolism of metafolin, interactions between medicaments which inhibit dihydrofolate reductase, such as, for example, methotrexate and dihydrofolate reductase are not to be expected.

In order to provide an adequate supply of folate also to women suffering from methylenetetrahydrofolate reductase deficiency, EP 0898965 (Müller et al.) proposes the use of 5-methyl-(6S)-tetrahydrofolic acid or appropriate pharmaceutically acceptable salts as dietary supplement or as ingredient of medicaments. EP 1044975 A1 discloses inter alia stable crystalline salts of 5-methyl-(6S)-tetrahydrofolic acid and processes for their preparation.

It is known that a large proportion of pregnancies occurs shortly after discontinuing the contraceptive (Farrow et al., Human Reproduction Vol. 17, No., 10, pp. 2754-2761, 2002). If administration is irregular and unreliable, pregnancy may even occur during intake. It is likewise known that even after termination of additional folate administration a person can profit therefrom for a further 90 days or so (FDA Advisory Committee for Reproductive Health Drugs (ACRHD): The public health issues, including the safety and potential clinical benefit, associated with combining folic acid and an oral contraceptive into a single combination product. December 15, 2003; Summary Minutes, Question 4). However, it is a precondition for this that folic acid has been taken in sufficiently large amount in addition to the normal diet in a sufficiently long preceding period. This so-called tissue depot effect can be seen through elevated folate levels in the erythrocytes.

It is further known that low folate/high homocysteine levels are associated with multiple spontaneous

abortions (Merlen et al., Obstet. et Gynecol. 2000, 95: pp. 519-524).

The present invention is based on the object of
5 producing an oral contraceptive which, although able to prevent diseases caused by folate deficiency, at the same time is unable to mask the symptoms of vitamin B₁₂ deficiency. The invention is further based on the object of disclosing an administration regime which
10 ensures that the consumer of the pharmaceutical composition of the invention is reliably protected also for a certain time after discontinuation from disorders or malformations caused by folate deficiency, in particular from neural tube defects. Both these also
15 apply in the case of a homozygous or heterozygous polymorphism of methylenetetrahydrofolate reductase in the user, which adversely affects the utilizability of folic acid by the body and thus its biological activity to prevent neural tube defects.

20 The object is achieved according to the invention by a pharmaceutical composition comprising one or more progestogens and/or estrogens and 5-methyl-(6S)-tetrahydrofolate, and pharmaceutically acceptable
25 excipients and carriers.

The invention is based on the realization, which is surprising in relation to WO 03/070255, that treatment and prevention of disorders caused by folate deficiency
30 is possible even without masking symptoms of vitamin B₁₂ deficiency by administering solely 5-methyl-(6S)-tetrahydrofolate. Administration of vitamin B₁₂ is therefore no longer necessary in order to avoid the health risk described in WO 03/070255. Despite the
35 administration of 5-methyl-(6S)-tetrahydrofolate, a physician is able to diagnose and, where appropriate, treat vitamin B₁₂ deficiency.

In the case of existing vitamin B₁₂ deficiency it is, of

course, possible to administer vitamin B₁₂ in addition. The addition of further vitamins such as, for example, vitamin B₆ or vitamin B₂ is likewise optionally possible. The invention is further based on the
5 realization, which is surprising in relation to WO 03/070255, that, unlike the administration of folates or other tetrahydrofolates, use solely of 5-methyl-(6S)-tetrahydrofolate in a contraceptive enables, even in a case of homozygous or heterozygous
10 polymorphism of methylenetetrahydrofolate reductase, unlimited and adequate utilizability of the folate component by the body and thus its biological activity to prevent congenital malformations caused by folate deficiency.

15 5-Methyl-(6S)-tetrahydrofolate is synthesized metabolically (see Figure 1) from 5,10-methylene-(6R)-tetrahydrofolate. This biochemical reaction is catalyzed by the enzyme methylenetetrahydrofolate
20 reductase (MTHFR), of which various genetic mutations are known, some of which are manifested by restricted biological activity (MTHFR C677T polymorphism). 5-Methyl-(6S)-tetrahydrofolate is converted in a further step which is catalyzed by the enzyme
25 methionine synthase (MS) into tetrahydrofolate. This entails transfer of the 5-methyl group of 5-methyl-(6S)-tetrahydrofolate to the amino acid homocysteine (Hcy) which is thus converted into the amino acid methionine (Met). This vitamin B₁₂-dependent reaction is
30 also referred to as homocysteine methylation in homocysteine metabolism. 5-Methyl-(6S)-tetrahydrofolate occupies a special place in the group of reduced folates because 5-methyl-(6S)-tetrahydrofolate can be converted into tetrahydrofolate only by the
35 homocysteine methylation reaction. Tetrahydrofolate is the actual carrier molecule for one-carbon units of various oxidation states. In metabolism, 5-methyl-(6S)-tetrahydrofolate can be synthesized only from 5,10-methylene-(6R)-tetrahydrofolate and can be further

metabolized only by conversion into tetrahydrofolate. The first enzymatic reaction (MTHFR) is irreversible under physiological conditions, and the second enzymatic reaction (MS) is vitamin B₁₂-dependent, meaning that if there is a vitamin B₁₂ deficiency then 5-methyl-(6S)-tetrahydrofolate accumulates and cannot be metabolized further. This phenomenon is also known under the name methyl trap. Only 5-methyl-(6S)-tetrahydrofolate, but not any other oxidized and reduced folates such as folic acid, 7,8-dihydrofolate, (6S)-tetrahydrofolate, 5-formyl-(6S)-tetrahydrofolate, 10-formyl-(6R)-tetrahydrofolate, 5,10-methenyl-(6R)-tetrahydrofolate, 5,10-methylene-(6R)-tetrahydrofolate, 5-formimino-(6S)-tetrahydrofolate, displays this particular property. 5-Methyl-(6S)-tetrahydrofolate is the only naturally occurring folate which does not mask vitamin B₁₂ deficiency. This is of particular importance on use of 5-methyl-(6S)-tetrahydrofolate in combination with oral contraceptives and is an aspect of the present invention.

Progestogens which can be used in the pharmaceutical composition of the invention are the following substances: levonorgestrel, norgestimate, norethisterone, dyrogesterone, drospirenone, 3-beta-hydroxydesogestrel, 3-ketodesogestrel (= etonogestrel), 17-deacetylnorgestimate, 19-norprogesterone, acetoxypregnenolone, allylestrenol, amgestone, chlormadinone, cyproterone, demegestone, desogestrel, dienogest, dihydrogesterone, dimethisterone, ethisterone, ethynodiol diacetate, fluorogestone acetate, gastrinone, gestodene, gestrinone, hydroxymethylprogesterone, hydroxyprogesterone, lynestrenol (= lynoestrenol), mecirogestone, medroxyprogesterone, megestrol, melengestrol, nomegestrol, norethindrone (= norethisterone), norethynodrel, norgestrel (including d-norgestrel and dl-norgestrel), norgestrienone, normethisterone, progesterone, quingestanol, (17alpha)-17-hydroxy-11-

methylene-19-norpregna-4,15-dien-20-yn-3-one, tibolone, trimegestone, algestone acetophenide, nestorone, promegestone, 17-hydroxyprogesterone esters, 19-nor-17hydroxyprogesterone, 17alpha-ethynyltestosterone, 5 17alpha-ethynyl-19-nortestosterone, d-17beta-acetoxy-13beta-ethyl-17alpha-ethynylgon-4-en-3-one oxime or the compounds disclosed in WO 00/66570, especially tanaproget. Levonorgestrel, norgestimate, norethisterone, drospirenone, dydrogesterone are 10 preferred. Drospirenone is particularly preferred.

Suitable estrogens are ethinylestradiol, mestranol, quinestranol, estradiol, estrone, estrane, estriol, estetrol and conjugated equine estrogens. In this 15 connection ethinylestradiol, estradiol and mestranol are preferred, and ethinylestradiol is particularly preferred.

The amounts used according to the invention of the 20 respective progestogens and/or estrogens correspond to the amounts normally known in contraceptives.

These amounts are normally for example for the progestogens mentioned below:

25

	Drospirenone	0.5 - 5 mg
	Levonorgestrel	30 - 250 µg
	Norgestimate	180 - 250 µg
	Norethisterone acetate	0.5 - 1 mg
30	Cyproterone acetate	1 - 2 mg
	Desogestrel	20 - 150 µg
	Dienogest	2 - 3 mg
	Gestodene	60 - 75 µg
	Tibolone	2.5 mg

35

The preferred amount administered each day according to the present invention is for example 0.5 to 5 mg, particularly preferably 3 mg, of drospirenone.

The amount of estrogen used according to the invention is for instance for the estrogens mentioned below:

	Ethinylestradiol	10 - 50 µg
5	Estradiol	1 - 4 mg
	Mestranol	50 µg

The preferred amount administered each day according to the invention is for example 10 to 50 µg, particularly preferably 10 to 30 µg, very particularly preferably 20 to 30 µg, of ethinylestradiol.

Reference to 5-methyl-(6S)-tetrahydrofolates in the form according to the invention means the free acid form and pharmaceutically acceptable salts and modifications of 5-methyl-(6S)-tetrahydrofolic acid (N-[4-[[(2-amino-1,4,5,6,7,8-hexahydro-4-oxo-5-methyl-(6S)-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid).

Pharmaceutically acceptable salts are intended to be both pharmacologically and pharmaceutically acceptable. Such pharmacologically and pharmaceutically acceptable salts may be alkali metal or alkaline earth metal salts, preferably sodium, potassium, magnesium or calcium salts. The calcium salt is particularly preferred.

The amount used for example of the calcium salt, which is particularly preferred according to the invention, of 5-methyl-(6S)-tetrahydrofolic acid (metafolin) is between 0.1 and 10 mg, preferably 0.4 to 1 mg, particularly preferably 451 µg (equivalent to 400 µg of folic acid or 416 µg of 5-methyl-(6S)-tetrahydrofolic acid).

Crystalline modifications disclosed in EP 1044975 are preferably employed as modification of 5-methyl-(6S)-tetrahydrofolates.

It is optionally possible for vitamin B₆ or vitamin B₂ to be present. However, a corresponding addition is unnecessary to carry out the invention. Vitamin B₆ may be used in a dose between 1 mg and 5 mg, preferably
5 between 1 mg and 3 mg per day on normally dosed use. Vitamin B₂ can be employed in a dose between 1 mg and 5 mg, preferably between 1 mg and 2 mg per day on normally dosed use, and between 2 and 5 mg per day on high-dosed use.

10

The progestogens and/or estrogens are the substances with contraceptive efficacy in this case. 5-Methyl-(6S)-tetrahydrofolate is added as vitamin in order to prevent disorders and malformations caused by folate
15 deficiency without, however, at the same time masking the symptoms of vitamin B₁₂ deficiency which may be present. In addition, those women who, because of their reduced MTHFR enzymic activity (MTHFR C677T polymorphism), are capable of only restricted
20 metabolism of folic acid, but also of reduced folates, also profit from 5-methyl-(6S)-tetrahydrofolate.

In the preferred variant of the present invention, the amount of drospirenone administered each day is 0.5 to
25 5 mg, preferably 3 mg, that of ethinylestradiol is 10 to 50 µg, preferably 10 to 30 µg, particularly preferably 20 to 30 µg. The calcium salt of 5-methyl-(6S)-tetrahydrofolic acid is present in an amount of from 0.1 to 10 mg, preferably 0.4 to 1 mg, particularly
30 preferably 451 µg (equivalent to 400 µg of folic acid) in this preferred variant of the present invention.

The formulation of pharmaceutical products based on the novel pharmaceutical composition takes place in a
35 manner known per se by processing the active ingredients with the carrier substances, fillers, substances influencing disintegration, binders, humectants, lubricants, absorbents, diluents, masking flavors, colorants and so on which are used in

pharmaceutical technology, and converting into the desired administration forms, which also include slow-release forms.

5 In medicaments of the invention it is possible for the estrogen and the progestogen, and the 5-methyl-(6S)-tetrahydrofolate, to be present in joint dosage units. The estrogen with the progestogen on the one hand, and the 5-methyl-(6S)-tetrahydrofolate on the other hand,
10 may, however, also be formulated in separate dose units.

(Both vitamin B₁₂ and 5-methyl-(6S)-tetrahydrofolate are unstable in the presence of atmospheric oxygen and
15 humidity. On attempting to formulate ethinylestradiol and vitamin B₁₂ together it was found that these two substances are incompatible with one another. Measurements of the incompatibilities between the intended formulation ingredients were carried out by a
20 thermoanalytical method (DSC, differential scanning calorimetry). This allows incompatibilities to be recognized through low enthalpies of fusion and melting temperatures. These are caused for example by a reduced proportion of crystalline substance and the increase in
(25 impurities. In the determination, binary mixtures of excipients or active ingredients in each case with vitamin B₁₂ were investigated, and the compatibility was examined under the influence of various gases and temperatures. Vitamin B₁₂ showed a strong interaction
30 with ethinylestradiol in the described investigations. The results of the incompatibility measurements may be found in Table 1.

Table 1: Summary of the compatibility investigation

Substance	Compat- ibility	Type of compatibility	Comments
Drospirenone	+	mainly good	O ₂ -sensitive
Ethinylestradiol	---	strong interaction	very O ₂ - sensitive
Ethinylestradiol β-cyclodextrin complex	+	mainly good below 60°C	O ₂ -sensitive, moisture- sensitive
Lactose	+	mainly good below 60°C	O ₂ -sensitive, moisture- sensitive
Corn starch	++	good below 60°C	O ₂ -sensitive, moisture- sensitive
Modified corn starch	++	good below 60°C	O ₂ -sensitive, moisture- sensitive
Polyvinylpyrrolidone	+	mainly good below 60°C	O ₂ -sensitive, moisture- sensitive
Magnesium stearate	++/-	indifferent, good below 60°C	O ₂ -sensitive, moisture- sensitive
Hydroxypropylmethyl- cellulose	++	good below 60°C	O ₂ -sensitive, moisture- sensitive
Hydroxypropyl- cellulose	++	good below 60°C	O ₂ -sensitive, moisture- sensitive
Maltodextrin	+/--	indifferent, good below 60°C	O ₂ -sensitive, moisture- sensitive
Polyethylene glycol 6000	-	interaction with moisture	O ₂ -sensitive, moisture- sensitive
Coating mix	+	mainly good below 60°C	O ₂ -sensitive, moisture- sensitive

Key:

- 5 ++ good compatibility expected below the stated
 temperature
- + compatibility below the stated temperature
- ++/- indifferent compatibility, possibly good
 compatibility below the stated temperature

+/-- indifferent compatibility, appears to be
compatible below the stated temperature
-(---) (strong) interaction, incompatible

5 Polyvinylpyrrolidone (PVP) is particularly suitable
because of its wetting properties for hormone
formulations (Moneghini et al., Int J Pharm 175, 1998,
177-183). However, formulation of 5-methyl-(6S)-
tetrahydrofolate with PVP increases the rate of
10 degradation of 5-methyl-(6S)-tetrahydrofolate (compare
Table 2 and 3; process 3).

(A further object on which this application was based
and which is achieved by the present invention is
15 therefore to make stable formulation of
ethinylestradiol in the presence of 5-methyl-(6S)-
tetrahydrofolate and optionally of vitamin B₁₂ possible.

It has been found that incompatibility between
20 ethinylestradiol and vitamin B₁₂ can surprisingly be
prevented by employing the ethinylestradiol in the
formulation as ethinylestradiol-beta-cyclodextrin
complex (ethinylestradiol as β -cyclodextrin clathrate;
for preparation, compare WO 02/49675).

(25 Corresponding formulations of the invention are
described in Example 1 (compare composition A, B and
D).

30 They comprise inter alia a mixture of corn starch and
modified corn starch. Starch consists of amylose and
amylopectin. Both are polysaccharides based on α -
glucose units. However, it is also possible to use
instead of corn starch in pharmaceutical formulations
35 for example rice starch, potato starch or wheat starch.
The starch is employed swollen, suspended or dissolved
as binding liquid or as solid. It may be unmodified or
partly modified. The corn starch which is preferably
used according to the invention has the empirical

formula $(C_6H_{10}O_5)_n$ with $n = 300-1000$. Its molecular weight is 50 000-160 000.

5 The starch used in pharmaceutical formulations serves only in part as pure filler. It is used otherwise as binder. 1-5%, preferably -1.8-3% of the tablet weight are to be added according to the invention as binder in the form of corn starch. Besides the corn starch, it is also possible to employ starch, a starch compound such
10 as maltodextrin, or cellulose derivatives such as, for example, carboxymethylcellulose, ethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose or methylcellulose as binder. It is preferred according to the invention to use low-substituted cellulose
15 derivatives. These have a viscosity of 1-20 mPas in a 2 percent aqueous solution. Derivatives with a viscosity of 2-20 mPas are preferred according to the invention, and those with a viscosity of 3-6 mPas are particularly preferred.

20 Part of the corn starch used in the formulation preferred according to the invention may be replaced by low-substituted hydroxypropylcellulose (HPC) in a concentration of 0.5-5% (w/w), preferably 1-3% (w/w),
25 particularly preferably 2% (w/w). In the present case, the hydroxypropylcellulose has low substitution when no fewer than 5% and no more than 16% of its hydroxyl groups are esterified or etherified.

30 Table 2 shows the 5-methyl-(6S)-tetrahydrofolate content per tablet in % based on the specified content of 100% as a function of the binder used immediately after preparation. The 5-methyl-(6S)-tetrahydrofolate content shown was measured in the content uniformity
35 test (CUT). The investigated formulation was prepared (process 2) by mixing the ingredients, granulating with the part of the corn starch used as binder, absorbing the 5-methyl-(6S)-tetrahydrofolate after completion of the granulation process, renewed mixing and tableting.

By comparison therewith, polyvinylpyrrolidone was added as binder instead of corn starch to the formulation by process 3. The 5-methyl-(6S)-tetrahydrofolate content in the formulation prepared by process 3 is lower.

5

Table 2: Metafolin content as a function of the binder immediately after preparation

	Metafolin content absorption, PVP (process 3)	Metafolin content absorption, corn starch (process 2)
Average	90.5%	96.1%

10 Table 3 shows the 5-methyl-(6S)-tetrahydrofolate content as a function of the binder used after storage at defined temperatures and humidity for one month. The tendency, evident from Table 2, for 5-methyl-(6S)-tetrahydrofolate formulated with PVP to be less stable
15 is confirmed in particular on storage under conditions at 40°C and 75% relative humidity (rH).

Table 3: Metafolin content as a function of the binder after storage

20

	25°C/60% rH absorption PVP (process 3)	25°C/60% rH absorption corn starch (process 2)	40°C/75% rH absorption PVP (process 3)	40°C/75% rH absorption corn starch (process 2)
Vials open	89.5%	92.1%	37.7%	67.7%

An oral formulation is normally prepared by granulation, tableting and film-coating. However, 5-methyl-(6S)-tetrahydrofolate is, because of its
25 sensitivity to oxygen and moisture, degraded even during the granulation. The further degradation of 5-methyl-(6S)-tetrahydrofolate during storage is, however, particularly noteworthy. In a formulation in which - as usual - all the components of the
30 medicament, including 5-methyl-(6S)-tetrahydrofolate,

are mixed first and only then granulated, the residue remaining after a storage time of one month at 40°C and 75% relative humidity in closed vials was only just 60% (compare Table 5) of the originally employed 5-methyl-
5 (6S)-tetrahydrofolate. The losses during the granulation process can be reduced by absorbing the 5-methyl-(6S)-tetrahydrofolate only after completion of the granulation process. Dry admixture during the preparation thus leads to stabilization of the
10 5-methyl-(6S)-tetrahydrofolate. However, in addition, this also surprisingly has the effect of further stabilization during storage. The 5-methyl-(6S)-tetrahydrofolate content in a formulation prepared by later absorption is above 90% with identical storage
15 times under identical conditions (compare Table 5).

Table 4 shows the 5-methyl-(6S)-tetrahydrofolate content per tablet in % as a function of the preparation process used immediately after preparation.
20 The difference between process 1 and process 2 derives from the time at which the 5-methyl-(6S)-tetrahydrofolate was added during the preparation of the investigated tablet. In process 1, the 5-methyl-(6S)-tetrahydrofolate was present in the mixture even
25 during the granulation, whereas in process 2 it was absorbed only after the granulation. The 5-methyl-(6S)-tetrahydrofolate content in the formulation prepared by process 1 is distinctly lower.

30

Table 4: Metafolin content as a function of the preparation process immediately after preparation

	Metafolin content granulation (process 1)	Metafolin content absorption (process 2)
Average	88.5%	96.1%
Distribution coefficient	6.1	2.5

Table 5 shows the 5-methyl-(6S)-tetrahydrofolate content as a function of the preparation process used after storage for one month at defined temperatures and humidity. The tendency, evident from Table 4, for 5-methyl-(6S)-tetrahydrofolate added before the granulation to be less stable is confirmed in particular on storage under conditions of 40°C and 75% relative humidity (rH).

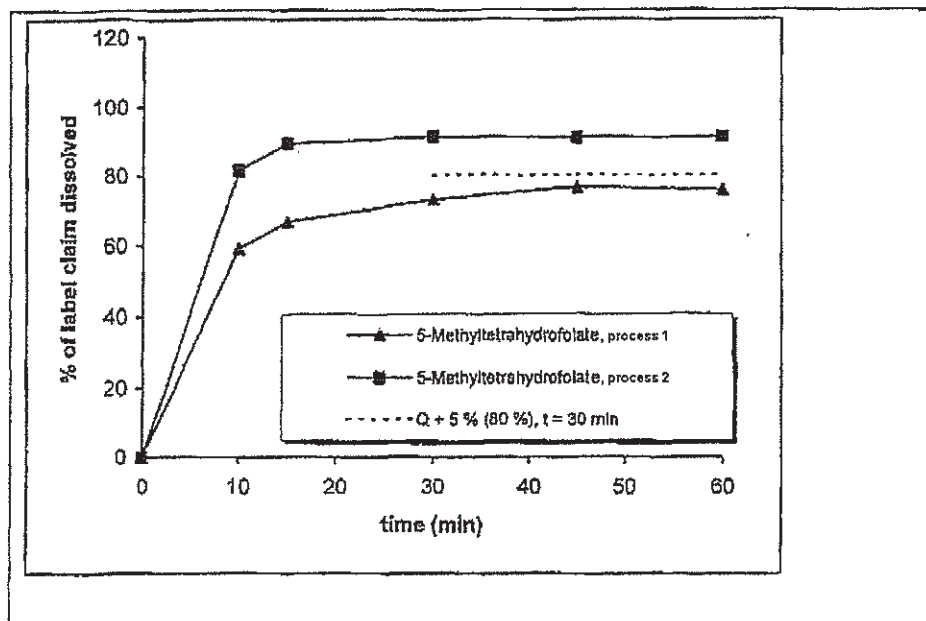
Table 5: Metafolin content as a function of the preparation process after storage

	25°C/60% rH granulation (process 1)	25°C/60% rH absorption (process 2)	40°C/75% rH granulation (process 1)	40°C/75% rH absorption (process 2)
Vials open	63.2%	92.1%	43.4%	67.7%
Vials closed	74.5%	92.5%	58.9%	90.1%

It is known that release takes place more slowly with dry admixture than in the case of granulation. However, it was surprisingly found that dry admixture of the 5-methyl-(6S)-tetrahydrofolate does not delay release, but in fact accelerates it. For this purpose, the tablets were investigated in an in vitro dissolution test using a USP paddle apparatus at 50 rpm and 37°C in a 0.03 percent aqueous ascorbic acid solution. Table 6 shows the results of the in vitro dissolution tests.

Table 6: Dissolution in %

Time [min]	5-Methyl-(6S)- tetrahydrofolate process 1 dissolution [%]	5-Methyl-(6S)- tetrahydrofolate process 2 dissolution [%]
0	0	0
10	59.2	81.4
15	66.8	89.3
30	73.1	91.3
45	76.7	91.1
60	75.8	91.2



5

Regular intake of the pharmaceutical composition of the invention with the particularly preferred dose of 451 μg of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid per day leads to an increase in the folate concentrations in the serum and erythrocytes until a steady state is reached. The corresponding erythrocyte folate invasion kinetics are described by half-life of from 6 to 10 weeks. On the basis of this half-life, about 97% of the steady-state erythrocyte

10

folate level can be expected to be reached after about 5 half-lives (corresponding to about 30 to 50 weeks). If daily intake of the pharmaceutical composition of the invention is continued, the erythrocyte folate levels remain in the region of the steady-state concentrations. After discontinuation of the pharmaceutical composition of the invention, the erythrocyte folate levels slowly fall with a half-life likewise of about 6 to 10 weeks. The erythrocyte folate levels thus remain even without further continuation of intake of the pharmaceutical composition of the invention for several weeks in a range above the limit of 906 nmol/l which is generally regarded as sufficient to prevent neural tube defects. The product of the invention thus ensures a reduction in the risk of disorders caused by folate deficiency and congenital malformations caused by folate deficiency, even after termination of long-term intake of the medicament of the invention ("pill").

The use of 5-methyl-(6S)-tetrahydrofolate, one or more estrogens and/or progestogens, and optionally vitamin B₆ and/or vitamin B₂, and pharmaceutically acceptable excipients and carriers for producing a medicament for reducing the risk of disorders caused by folate deficiency and congenital malformations caused by folate deficiency for at least 8 weeks after termination of previous long-term and continual intake of this medicament is also according to the invention.

Likewise according to the invention is a kit comprising at least 20 daily dose units comprising the medicament of the invention and at least one daily dose unit comprising 5-methyl-(6S)-tetrahydrofolate, and optionally vitamin B₁₂, vitamin B₆ and/or vitamin B₂, where the number of all the dose units present in the kit is at least 28, and the dose units are disposed so that first the dose units comprising the medicament of the invention, and then the dose units comprising

neither estrogen nor progestogen, are to be taken. It is also possible in the case of the first-mentioned at least 20 daily dose units comprising the medicament of the invention for the 5-methyl-(6S)-tetrahydrofolate to
5 be formulated separately and to be disposed spatially as additional dose units such that joint intake of both dose units is evident from this disposition.

Further developments of the invention for different
10 kits are reflected in claims 18 to 22, 38, 39 and 40.

It is also possible in particular, according to claims 43 to 50, for the medicament of the invention to be administered in a so-called extended regime. By this is
15 meant continuous administration of the medicament for more than 28 days, the extended cycle of use being completed by administration for 1 to 7 days of dose units exclusively comprising 5-methyl-(6S)-tetrahydrofolate or by intake of 1 to 7 placebos (dose
20 units active agent) or 1 to 7 blank pill days (no administration of any dose unit).

The following examples serve to explain the subject matter of the invention in more detail without wishing
25 to restrict it thereto.

Example 1:

The composition of tablets (80 mg) of the invention can be found in Table 7.

5

Table 7: Composition of tablets of the invention

Ingredient	Amount			
	Composition A	B	C	D
Drospirenone	3 mg	3 mg	--	3 mg
Ethinylestradiol*	0.03 mg	0.02 mg	--	0.03 mg
Metafolin	0.451 mg	0.451 mg	0.451 mg	0.451 mg
Vitamin B12	--	--	--	0.1 mg
Lactose monohydrate	to 80 mg	to 80 mg	to 80 mg	to 80 mg
Corn starch	16.40 mg	16.40 mg	16.40 mg	16.40 mg
Corn starch**	2 mg***	2 mg***	2 mg***	2 mg***
Modified corn starch	9.60 mg	9.60 mg	9.60 mg	9.60 mg
Magnesium stearate	0.80 mg	0.80 mg	0.80 mg	0.80 mg

10 *: optionally as ethinylestradiol-beta-cyclodextrin complex; the stated amount refers in this case to uncomplexed ethinylestradiol. If the ethinylestradiol-beta-cyclodextrin complex is used, about ten times the amount is to be employed. This is because the ethinylestradiol content in the β -cyclodextrin complex is about 9.5 to 12.5% (compare WO 02/49675).

15 **: the part of the corn starch identified by ** can be replaced by an alternative binder such as, for example, 1.6 mg of low-substituted hydroxypropyl-cellulose.

20 ***: the amount of corn starch ** employed as binder may also be for example 1.8 mg.

25 The oral formulation is produced by mixing the abovementioned ingredients, granulating with the part of the corn starch used as binder, absorbing the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid after completion of the granulation process, renewed

mixing, tableting and film-coating.

Example 2:

5 Blood is taken at 8-week intervals from 80 healthy
young women of childbearing age, and the erythrocyte
folate level is determined using a validated
microbiological, immunological or instrumental (e.g.
HPLC, LC-MS/MS) method or a suitable combination of
10 these methods.

8 Weeks after the first blood sampling (screening
phase),

451 µg of the calcium salt of 5-methyl-(6S)-
15 tetrahydrofolic acid each day
is administered over a period of 40 weeks or,
alternatively:

3 mg of drospirenone, 30 µg of ethinylestradiol
and 451 µg of the calcium salt of 5-methyl-(6S)-
20 tetrahydrofolic acid is administered
simultaneously on each of the first 21 days of the
respective cycle (tablet of composition A in
Example 1). Administration of 451 µg of the
calcium salt of 5-methyl-(6S)-tetrahydrofolic acid
25 is continued for 7 days in a phase immediately
subsequent thereto (composition C). 3 mg of
drospirenone, 30 µg of ethinylestradiol and 451 µg
of the calcium salt of 5-methyl-(6S)-
tetrahydrofolic acid (composition A) are again
30 administered for a further 21 days (second cycle),
and only 451 µg of the calcium salt of 5-methyl-
(6S)-tetrahydrofolic acid (composition C) are
administered for a further 7 days, and so on
(medication phase).

35 5-Methyl-(6S)-tetrahydrofolate is no longer
administered after 48 weeks. Alternatively,
drospirenone and ethinylestradiol can be administered
further for a further 40 weeks or likewise be

discontinued.

The last blood sample is taken after 88 weeks. The drop-out rate may be up to 50% because of the long-term nature of the study.

Example 3:

Blood is taken at 8-week intervals from 80 healthy young women of childbearing age, and the erythrocyte folate level is determined using a validated microbiological, immunological or instrumental (e.g. HPLC, LC-MS/MS) method or a suitable combination of these methods.

8 Weeks after the first blood sampling, 3 mg of drospirenone, 20 µg of ethinylestradiol and 451 µg of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (composition B) is administered simultaneously in each case in the first 24 days of the respective cycle for a period of 40 weeks. In a phase immediately subsequent thereto, administration of 451 µg of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid is continued for 7 days (composition C). For a further 21 days (second cycle), 3 mg of drospirenone and 20 µg of ethinylestradiol and 451 µg of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (composition B) are again administered, and only 451 µg of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (composition C) are administered for a further 7 days, and so on.

5-Methyl-(6S)-tetrahydrofolate is no longer administered after 48 weeks, while drospirenone and ethinylestradiol is administered further for a further 40 weeks or likewise discontinued.

The last blood sample is taken after 88 weeks. The drop-out rate may be up to 50% because of the long-term nature of the study.

The initial erythrocyte folate level in the subjects is about 500 to 700 nmol/l, depending on the eating habits, but is in every case below 906 nmol/l. This value rises on administration of the pharmaceutical composition of the invention in subsequent days, while the eating habits remain the same, and reaches a value of about 906 nmol/l after only 6 to 8 weeks - i.e. after the second cycle. After continuous administration for at least 30 weeks (corresponding to five times the lower limit of the half-life), while the eating habits remain the same, the erythrocyte folate level reaches about 1200 to 1600 nmol/l (steady state). After termination of the administration of 5-methyl-(6S)-tetrahydrofolate, the erythrocyte folate level falls continuously. Starting from an average steady state concentration of 1400 nmol/l and with the eating habits remaining the same, the erythrocyte folate level is expected to fall below 906 nmol/l, and thus the minimum concentration in erythrocytes generally sufficient to prevent neural tube defects, in the eleventh to thirteenth week after discontinuation of the pharmaceutical composition of the invention.

Example 4: Long-term folate study

180 healthy young women of child-bearing age (half of whom receive a diet fortified with folic acid) have blood taken at intervals of 2 weeks, and the erythrocyte folate level is determined using a validated microbiological, immunological or instrumental (e.g. HPLC, LC-MS/MS) method or a suitable combination of these methods.

8 weeks after the first taking of blood, a first group of 90 women receives over a period of 24 weeks

administration of simultaneously 3 mg of drospirenone, 30 µg of ethinylestradiol and 451 µg of the calcium salt of 5-methyl-(6S)-tetrahydro-

folic acid in each of the first 21 days of the respective cycle. In a phase directly subsequent thereto, administration of 451 μ g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid is continued for 7 days. For a further 21 days (second cycle), again 3 mg of drospirenone, 30 μ g of ethinylestradiol and 451 μ g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid, and for a further 7 days only 451 μ g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid, are administered, and so on (medication phase).

(A group of 90 women receive administration of 3 mg of drospirenone, 30 μ g of ethinylestradiol and 400 μ g of folic acid according to the same administration scheme as control group.

The last blood is taken in both cases after 24 weeks. This is followed by an observation period of 20 weeks in which the contraceptive product Yasmin® is administered for 20 weeks, i.e. in each of the first 21 days of the respective cycle, 3 mg of drospirenone and 30 μ g of ethinylestradiol are administered simultaneously; directly subsequent thereto, no active substance is administered (placebos or no administration) for 7 days. The dropout rate may be up to 30%.

(The initial erythrocyte folate level in the subjects is below 906 nmol/l. This value increases in subsequent days, if the eating habits remain the same, with administration of the pharmaceutical composition of the invention and, in most of the women, reaches a value of about 906 nmol/l after 6 to 8 weeks. After continuous administration for 24 weeks and with the eating habits remaining the same, an erythrocyte folate level is reached in both groups which shows equivalence between the two treatment groups (bioequivalence criterion 80-125%). After termination of administration of 5-methyl-

(6S)-tetrahydrofolate, the erythrocyte folate level falls continuously. It is ascertained when the erythrocyte folate level falls below the acknowledged threshold of 906 nmol/l, which is generally regarded as
5 sufficient to avoid neural tube defects.

Such an adequate erythrocyte folate level is still shown by most of the women in the first group 3 months after termination of intake.

Claims

1. A method for formulating a medicament comprising:

a daily dose of 0.4 to 1 mg of 5-methyl-(6S)-tetrahydrofolate;
one or more estrogens and/or progestogens; and
one or more pharmaceutically acceptable excipients/carriers
in the absence of vitamin B12,

wherein the 5-methyl-(6S)-tetrahydrofolate is absorbed only after granulation.

2. A method for formulating a medicament according to Claim 1, wherein the one or more estrogens is selected from the group consisting of ethinylestradiol, mestranol, quinestranol, estradiol, estrone, estrane, estriol, estetrol and conjugated equine estrogens.

3. A method for formulating a medicament according to Claim 1 or Claim 2, wherein the one or more progestogens is selected from the group consisting of levonorgestrel, norgestimate, norethisterone, dydrogesterone, drospirenone, 3-beta-hydroxydesogestrel, 3-ketodesogestrel (= etonogestrel), 17-deacetylnorgestimate, 19-norprogesterone, acetoxypregnenolone, allylestrenol, amgestone, chlormadinone, cyproterone, demegestone, desogestrel, dienogest, dihydrogesterone, dimethisterone, ethisterone, ethynodiol diacetate, fluorogestone acetate, gastrinone, gestodene, gestrinone, hydroxymethylprogesterone, hydroxyprogesterone, lynestrenol (lynoestrenol), necirogestone, medroxyprogesterone, megestrol, melengestrol, nomegestrol, norethindrone (norethisterone), norethynodrel, norgestrel, d-norgestrel, dl-norgestrel, norgestrienone, normethisterone, progesterone, quingestanol, (17alpha)-17-hydroxy-11-methylene-19-norpregna-4,15-dien-20-yn-3-one, tibolone, trimegestone, algestone-acetophenide, nestorone, promegestone, 17-hydroxyprogesterone esters, 19-nor-17hydroxyprogesterone, 17alpha-ethynyltestosterone, 17alpha-ethynyl-19-nortestosterone, d-17beta-acetoxy-13beta-ethyl-17alpha-ethynylgon-4-en-3-one oxime and tanaproget.

4. A method for formulating a medicament according to any one of Claims 1 to 3, wherein the medicament comprises a crystalline calcium

salt of 5-methyl-(6S)-tetrahydrofolic acid.

5. A method for formulating a medicament according to Claim 1, wherein the medicament comprises 5-methyl-(6S)-tetrahydrofolate, drospirenone and ethinylestradiol.

6. A method for formulating a medicament according to Claim 5, wherein the medicament comprises a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid at a daily dose of 451 μ g.

7. A method for formulating a medicament according to Claim 5 or Claim 6, wherein the medicament comprises a daily dose of 3 mg of drospirenone.

8. A method for formulating a medicament according to any one of Claims 5 to 7, wherein the medicament comprises a daily dose of from 10 to 50 μ g of ethinylestradiol.

9. A method for formulating a medicament according to any one of Claims 5 to 7, wherein the medicament comprises a daily dose of from 10 to 30 μ g of ethinylestradiol.

10. A method for formulating a medicament according to any one of Claims 5 to 7, wherein the medicament comprises a daily dose of 20 μ g of ethinylestradiol.

11. A method for formulating a medicament according to any one of Claims 5 to 7, wherein the medicament comprises a daily dose of 30 μ g of ethinylestradiol.

12. A method for formulating a medicament according to Claim 5, wherein the medicament comprises:

a daily dose of 451 μ g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid;
a daily dose of 3 mg of drospirenone; and
a daily dose of 20 μ g of ethinylestradiol.

13. A method for formulating a medicament according to Claim 5, wherein the medicament comprises:

a daily dose of 451 μ g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid;
a daily dose of 3 mg of drospirenone; and
a daily dose of 30 μ g of ethinylestradiol.

14. A medicament formulated by the method of any one of Claims 1 to 13.