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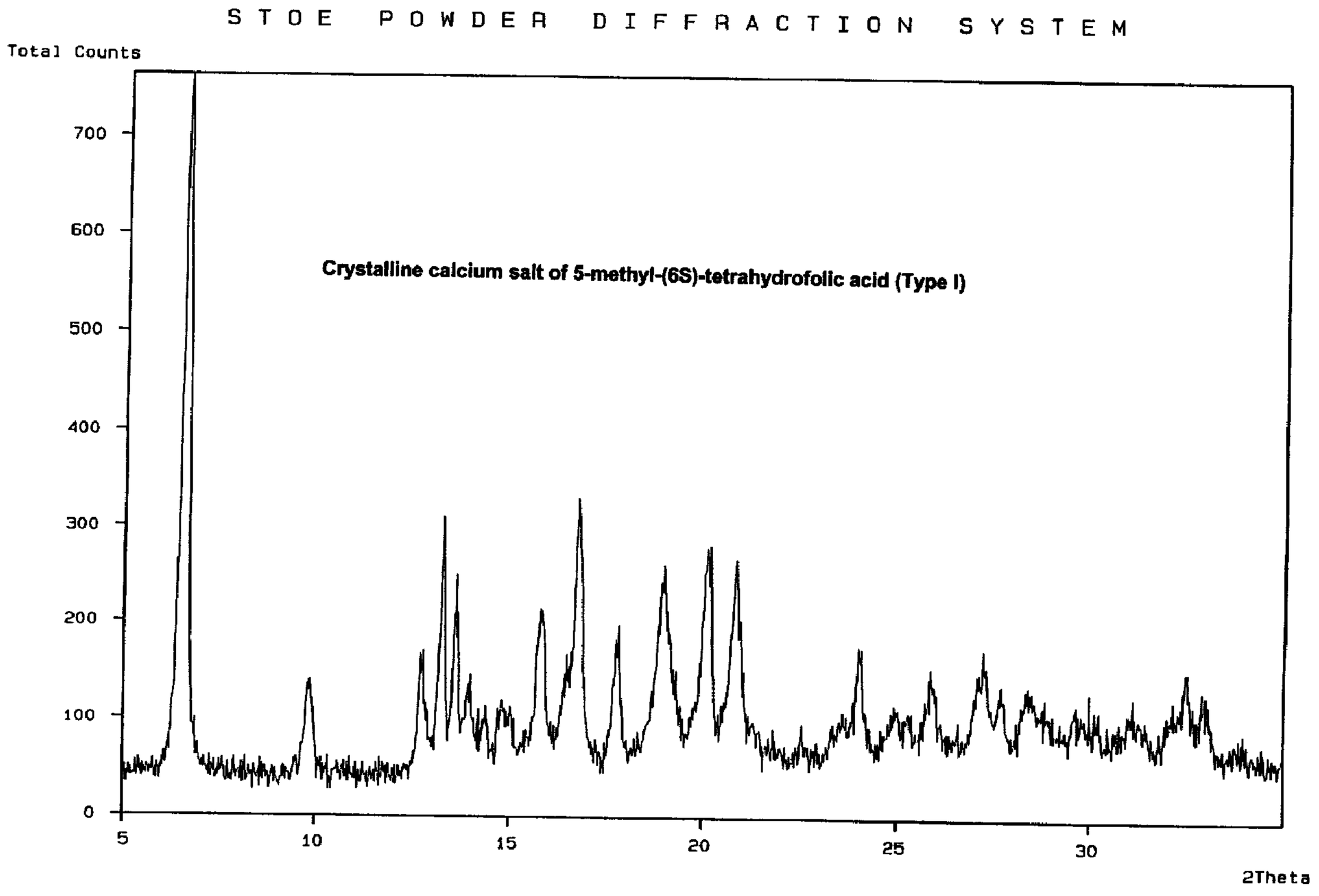
(72) Inventeurs/Inventors:
MULLER, HANS RUDOLF, CH;
MOSER, RUDOLF, CH;
EGGER, THOMAS, CH

(73) Propriétaire/Owner:
EPROVA AG, CH

(74) Agent: RICHES, MCKENZIE & HERBERT LLP

(54) Titre : SELS CRISTALLINS STABLES DE L'ACIDE 5-METHYLTETRAHYDROFOLIQUE
(54) Title: STABLE CRYSTALLINE SALTS OF 5-METHYLTETRAHYDROFOLIC ACID



(57) Abrégé/Abstract:
This invention relates to stable crystalline salts of 5-methyl-(6R,S)-, -(6S)- and -(6R)-tetrahydrofolic acid, to methods of producing these salts and to the use thereof use as a constituent for the production of drugs or as a food additive, and to preparations containing these salts.

Abstract

This invention relates to stable crystalline salts of 5-methyl-(6R,S)-, -(6S)- and -(6R)-tetrahydrofolic acid, to methods of producing these salts and to the use thereof use as a constituent for the production of drugs or as a food additive, and to preparations containing these salts.

Stable crystalline salts of 5-methyltetrahydrofolic acid

This invention relates to crystalline salts of N-[4-[[(2-amino-1,4,5,6,7,8hexahydro-4-oxo-5-methyl-(6S)-, -(6R)- and -(6R,S)-pteridinyl)methyl]amino]benzoyl-L-glutamic acid (hereinafter called salts of 5-methyltetrahydrofolic acid), to the use thereof, and to a method of producing them.

Tetrahydrofolates are predominantly used as 5-formyltetrahydrofolic acid and the salts thereof (leucovorin) or as 5-methyltetrahydrofolic acid and the salts thereof, for the treatment of megaloblastic folic acid anaemia, as an antidote for increasing the compatibility of folic acid antagonists, particularly of aminopterin and methotrexate in cancer therapy ("antifolate rescue"), for increasing the therapeutic effect of fluorinated pyrimidines and for the treatment of autoimmune diseases such as psoriasis and rheumatoid arthritis, for increasing the compatibility of certain antiparasitic formulations, for instance trimethoprim-sulfamethoxazole, and for reducing the toxicity of dideazatetrahydrofolates in chemotherapy. 5-methyltetrahydrofolic acid is used in particular as a drug and as a food additive, as a vitamin preparation, for the prevention of neural tube defects, for the treatment of depressive illnesses, and for influencing the homocysteine level.

5-methyltetrahydrofolic acid and salts thereof are extremely unstable, and in particular are highly susceptible to oxidation [see also A.L. Fitzhugh, Pteridines 4 (4), 187-191 (1993) in this respect] and are therefore difficult to produce at a level of purity which is acceptable for a pharmaceutical active ingredient or a food additive.

Various methods, such as excluding oxygen as completely as possible or the addition of antioxidants such as ascorbic acid or reduced L-glutathione, have been employed in order to overcome the instability of 5-methyltetrahydrofolic acid. However, it is scarcely possible completely to exclude oxygen during use, and even then this is only possible at very considerable cost, and the addition of antioxidants is likewise not always possible. Accordingly, it has not been possible hitherto to identify a commer-

cially feasible method which is suitable for the production of salts of 5-methyltetrahydrofolic acid which are satisfactorily stable and which are of high purity.

Surprisingly, it has now been found that salts of 5-methyltetrahydrofolic acid which exhibit high chemical purity and excellent stability can be obtained by crystallising the corresponding salt from a polar medium after subjecting the solution to thermal treatment at a temperature above 60°C. The highly crystalline salts of 5-methyltetrahydrofolic acid which are thus obtained are stable at room temperature, practically without limitations. They are suitable as a constituent or as a starting material for the production of drug forms or food additives.

Summary of Invention

Accordingly, the present invention relates to crystalline salts of 5-methyltetrahydrofolic acid. Alkaline earth salts, particularly the calcium salt, are preferably used as the salts of 5-methyltetrahydrofolic acid for crystallisation. These crystalline salts of 5-methyltetrahydrofolic acid exhibit a purity, which has never been achieved hitherto, of >98%, together with a stability, with respect to the initial value thereof and which has never been achieved hitherto, of >98% after storage for 6 months in air at 25°C and 60% relative atmospheric humidity. The crystalline calcium salts of 5-methyl-(6S)-tetrahydrofolic acid exist in four different crystalline modifications (Type I, Type II, Type III and Type IV) and exhibit sharp bands when subjected to X-ray powder diffraction measurements (Table 1 to Table 4 in this respect at the end of the disclosure). Selected 2 theta values for the different crystalline modifications are 6.5, 13.3, 16.8 and 20.1 (Type I); 5.3, 6.9, 18.7 and 21.1 (Type II); 6.8, 10.2, 15.4 and 22.5 (Type III); and 6.6, 15.9, 20.2 and 22.5 (Type IV). Crystalline calcium salts of 5-methyltetrahydrofolic acid have a content of water of crystallisation of at least 1 equivalent of water per 1 equivalent of 5-methyltetrahydrofolic acid. Thus the Type I modification typically contains ≥ 3 equivalents of water, the Type II modification typically contains ≤ 2 equivalents water and the Type III and Type IV modifications typically contain ≤ 5 equivalents of water.

Other salts of 5-methyl-(6R)-tetrahydrofolic acid and salts of 5-methyl-(6R,S)-tetrahydrofolic acid can likewise be obtained in highly crystalline form.

The present invention further relates to a method of producing highly crystalline salts of 5-methyltetrahydrofolic acid, which is characterised in that the corresponding salt of 5-methyltetrahydrofolic acid is crystallised. In this method, crystallisation of salts of 5-methyltetrahydrofolic acid is effected from a polar medium after thermal treatment at a temperature above 60°C, particularly above 85°C.

Substances which are particularly suitable as the polar medium include water or a mixture of water and an organic solvent which is miscible with water, such as water-soluble alcohols, e.g. methanol, ethanol, n-propanol, iso-propanol or ethylene glycol, a low molecular weight aliphatic water-soluble carboxylic acid e.g. formic acid, acetic acid or lactic acid, or water-soluble amides e.g. formamide, dimethylformamide, dimethylacetamide, 1-methylpyrrolidone, 2-methylpyrrolidone or 2-piperidinone. There are no particular restrictions with regard to the type of solvent used and with regard to the mixture ratio, since crystalline salts of 5-methyltetrahydrofolic acid generally exhibit solubilities which are lower than those of the corresponding amorphous forms.

Crystallisation is preferably effected from solutions. It is also possible to effect crystallisation from a suspension, however.

The different crystalline modifications can be converted into one another by further thermal treatments at temperatures above 60°C. Thus Type I, which is produced by crystallisation from a polar medium after thermal treatment at a temperature above 60°C, can be converted into Type II by drying under vacuum at 70°C, can be converted into Type III by thermal treatment at a temperature above 90°C, and can be converted into Type IV by thermal treatment at a temperature above 95°C. Type II can be converted into Type I again by treatment with water in a humidity cabinet at 90°C.

Crystallisation of the salts of 5-methyltetrahydrofolic acid occurs spontaneously or is effected by seeding with the corresponding crystalline salt of 5-methyltetrahydrofolic acid.

A suitable, preferred starting material for crystallisation is pure, amorphous or crystalline 5-methyl-(6S)- or -(6R)-tetrahydrofolic acid. Racemic 5-methyl-(6R,S) tetrahydrofolic acid can also be used, however, as can enriched 5-methyl-(6S)-, (6R)- or -(6R,S)-tetrahydrofolic acid.

By using amorphous or partly crystalline, optically pure 5-methyltetrahydrofolic acid or salts thereof as the starting material for crystallisation, essentially crystalline salts of 5-methyltetrahydrofolic acid of a purity which has never been achieved hitherto, together with a stability which has never been achieved hitherto, are obtained by the method described here.

The present invention also relates to the use of highly crystalline salts of 5-methyltetrahydrofolic acid as a constituent for the production of drugs or food additive substances or for the production of other tetrahydrofolic acid derivatives, since, on account of their excellent stability in solid form, crystalline salts of 5-methyltetrahydrofolic acid are of a very good quality which remains constant with time, practically without limits. The present invention also relates to preparations containing highly crystalline salts of 5-methyltetrahydrofolic acid. These preparations are produced by known methods. They are employed analogously to the use of known substances from the field of tetrahydrofolates, such as 5-formyltetrahydrofolic acid (leucovorin) for example.

In another aspect, the present invention provides a crystalline salt of 5-methyl-(6R,S)-, -(6S)- or -(6R)-tetrahydrofolic acid said crystalline salt having a water of crystallisation of at least one equivalent per equivalent of 5-methyltetrahydrofolic acid.

In another aspect, the present invention provides a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I) said crystalline salt having a water of crystallisation of at least one equivalent per equivalent of 5-methyltetrahydrofolic acid.

In another aspect, the present invention provides a method of producing crystalline salts of 5-methyl-(6R,S)-, -(6S)- and 5-methyl-(6R)-tetrahydrofolic acid, comprising subjecting a salt of 5-methyl-(6R,S)-, -(6S)- or -(6R)-tetrahydrofolic acid in a polar

medium to a thermal treatment, at a temperature above 60° C., and thereafter crystallising said salt from the resultant heated solution.

In another aspect, the present invention provides a method of producing 5-methyl-(6S)-tetrahydrofolic acids with 2 theta values of 5.3, 6.9, 18.7 and 21.1 (Type II) comprising drying sufficiently 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

In another aspect, the present invention provides a method of producing 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.8, 10.2, 15.4 and 22.5 (Type III) comprising subjecting to sufficient thermal treatment at above 90° C., a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

In another aspect, the present invention provides a method of producing 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.6, 15.9, 20.2, 22.5 (Type IV) comprising subjecting to sufficient thermal treatment at above 95° C., a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

Brief Description of the Drawings

The above-mentioned features as well as other features and objects of this invention and the matter of obtaining them will become more apparent and the invention itself will be best understood by reference to the following description of an embodiment of the invention taken in conjunction with the accompanying drawings in which:

Figure 1 is an X-Ray powder diffraction spectrum of crystalline calcium salts of 5-methyl-(6S)-tetrahydrofolic acid (Type I).

Figure 2 is an X-Ray powder diffraction spectrum of crystalline calcium salts of 5-methyl-(6S)-tetrahydrofolic acid (Type II).

Figure 3 is an X-Ray powder diffraction spectrum of crystalline calcium salts of 5-methyl-(6S)-tetrahydrofolic acid (Type III).

Figure 4 is an X-Ray powder diffraction spectrum of crystalline calcium salts of 5-methyl-(6S)-tetrahydrofolic acid (Type IV).

Figure 5 is an X-Ray powder diffraction spectrum of amorphous calcium salts of 5-methyl-(6S)-tetrahydrofolic acid.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and unless otherwise indicated, all parts and percentages are by weight.

Examples which illustrate the invention

The content of 5-methyltetrahydrofolic acid salt which is quoted in the examples was determined by HPLC in each case and is given as % area. The water content was determined by a Karl Fischer method.

Example 1 [stabilities]

In order to determine the stabilities of the crystalline salts of 5-methyltetrahydrofolic acid, the substances were stored, together with comparison specimens, in air at 25°C and at 60% relative humidity. The content of 5-methyltetrahydrofolic acid salt remaining was measured at periodic intervals and is given by comparison with the initial value.

	Time of storage in months					
	0	3	6	12	18	88
Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid	100%	98.6%	98.7%	99.1%	99.0%	97.8%
Amorphous calcium salt of 5-methyl-(6S)-tetrahydrofolic acid	100%			84.2%		

The crystalline salts of 5-methyltetrahydrofolic acid were still very light in colour even after an extended period of storage. In contrast thereto, the amorphous samples exhibited considerable discoloration, which occurred very rapidly.

Example 2 [X-ray powder plots]

X-ray powder plots (diffraction spectra) of these substances were recorded in order to characterise the structural properties (crystalline modifications) of the crystalline salts of 5-methyltetrahydrofolic acid.

The crystalline salts of 5-methyltetrahydrofolic acid exhibited spectra of good resolution, with sharp bands and low background effects. The spectra indicated highly crystalline constituents.

Examples of spectra are illustrated in Figure 1 (Type I), Figure 2 (Type II), Figure 3 (Type III) and Figure 4 (Type IV), and are presented in Table 1 (Type I), Table 2 (Type II), Table 3 (Type III) and Table 4 (Type IV). For comparison, a spectrum of an amorphous sample was also recorded under analogous conditions and is presented as Figure 5 (amorphous).

Selected 2 theta values for the different crystalline modifications of the crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid are listed below:

Type	**Selected 2 theta values**
Type I	6.5, 13.3, 16.8 and 20.1
Type II	5.3, 6.9, 18.7 and 21.1
Type III	6.8, 10.2, 15.4 and 22.5
Type IV	6.6, 15.9, 20.2 and 22.5

Example 3 [solubilities]

The solubility of the crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid is given in the following Table:

Type	Solubility at 20°C in	
	0.9% NaCl	water
Type I	1.6%	1.1%
Type II	5.8%	3.8%
Type III	1.5%	1.0%

Example 4 [amorphous calcium salt of 5-methyl-(6S)-tetrahydrofolic acid]

7.5 g 5-methyl-(6S)-tetrahydrofolic acid were introduced into 75 ml water at room temperature whilst passing N_2 into the batch, and the batch was adjusted to pH 12 with aqueous 30% sodium hydroxide solution. The clear solution which was thus obtained was adjusted to pH 7.5 with 37% hydrochloric acid and was treated with a solution of 7.15 g calcium chloride $6H_20$ in 11.7 ml water. The white suspension which was formed was stirred for 5 hours and was then filtered under suction at room temperature. The solid was washed with water and was dried under vacuum at 45°C.

5.8 g of a white, amorphous calcium salt of 5-methyl-(6S)-tetrahydrofolic acid were obtained, which had a content of 98.0% and a 6S fraction corresponding to 99.6%.

Even after treating this substance at 60°C in a humidity cabinet, no crystalline fractions could be determined either under a polarising microscope or by X-ray diffraction measurements.

Example 5 [crystalline calcium salt of 5-methyl-(6 R,S)-tetrahydrofolic acid]

70 g 5-methyl-(6R, S)-tetrahydrofolic acid were placed in a vessel in 780 ml water and the batch was adjusted to pH 7.5 with 45.2 g of 30% NaOH. The clear, slightly reddish solution was treated with a solution of 62.7 g calcium chloride $6H_20$ in 140 ml

water, and the solid was filtered off and washed with a little water. The crude product which was thus obtained was suspended in water and treated at 90°C for 24 hours.

74.0 g of a white, crystalline calcium salt of 5-methyl-(6R,S)-tetrahydrofolic acid was obtained, with a content of 99.1%.

Example 6 [crystalline calcium salt of 5-methyl-(6R)-tetrahydrofolic acid]

16.5 g 5-methyl-(6R)-tetrahydrofolic acid were placed in a vessel in 100 ml water at 92°C with 50 g calcium chloride $6H_20$. The clear, slightly yellowish suspension was stirred for 10 minutes at 91°C, and the solid was filtered off, washed with a little water and dried at 35°C under vacuum.

15.4 g of a light beige crystalline calcium salt of 5-methyl-(6R)-tetrahydrofolic acid were obtained, with a content of 97.9% and a water content of 7.8%.

Example 7 [Type I]

130 kg water were placed in a vessel and 12.8 kg 5-methyl-(6S)-tetrahydrofolic acid were introduced. The pH was adjusted to 11.6 with about 9.1 kg of 30% NaOH, and was then adjusted to 7.6 with about 1.9 kg of 37% hydrochloric acid. A suspension containing 0.3 kg carbon and 0.3 kg Cellflock was added to the clear solution. The solid was filtered off and washed with 13 litres of water. The filtrate was treated with a solution containing 8.3 kg calcium chloride $2H_20$, heated to 90°C and stirred for 30 minutes. The product was filtered hot and was washed with 2 x 20 kg water. The moist crude product which was thus obtained was slurried in 115 litres of water, heated to 90°C, immediately filtered hot, washed with 2 x 20 kg water, and dried at 40°C under vacuum.

11.6 kg of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I) were obtained, which had a purity of 99.0% and a water content of 14.5%.

Example 8 [Type I]

1600 ml water were placed in a vessel and 194 g 5-methyl-(6S)-tetrahydrofolic acid were introduced. The pH was adjusted to 7.0 with about 80 ml of 30% NaOH. A suspension containing 20 g carbon and 20 g Cellflock in 190 ml water was added to the clear solution. The solid was filtered off and washed with water. The filtrate was treated with 950 ml of a 5.5 M calcium chloride solution, heated to 90°C and stirred for 60 minutes. The product was filtered hot, washed with water, and dried at 45°C under vacuum.

156.2 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I) were obtained, with a purity of 99.7% and a 6S fraction of 99.9%.

Example 9 [Type 1 and conversion into Type II]

554 g water were placed in a vessel and 53.1 g 5-methyl-(6S)-tetrahydrofolic acid were introduced. The pH was adjusted to 7.5 with 30% NaOH. 1.3 g carbon, 1.3 g Cellflock and 19.5 g water were added to the clear solution. The suspension was filtered and the solid was washed with 55 ml water. The filtrate was treated with a solution of 52.0 g calcium chloride $6H_20$ in 84.6 g water, and was heated to 90°C and seeded with 100 mg of the crystalline calcium salt of 5-methyltetrahydrofolic acid. After crystallisation had occurred, the product was filtered hot at 90°C and was washed with 2 x 103 g water. The moist crude product which was thus obtained was slurried in 480 ml water, heated to 90°C, immediately filtered hot, washed as above, and dried at 45°C under vacuum.

47.5 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I) were obtained, with a purity of 98.8% and a water content of 12.2%.

This Type I modification could be converted into the Type II modification with a water content of 5.0% by drying it at 70°C under vacuum for 30 minutes.

Example 10 [Type III]

15.8 g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid were heated to 95°C in 140 ml water whilst passing N_2 through the batch. After 30 minutes at 95°C the white suspension was filtered hot under suction, and the solid was washed with water and dried at 35°C under vacuum.

14.0 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type III) was obtained, with a content of 98.9% and a 6S fraction of 99.9%.

Example 11 [Type IV]

20.0 g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid were heated to 100°C in 180 ml water whilst passing N_2 through the batch. After 30 minutes at 100°C the white suspension was filtered hot under suction, and the solid was washed with water and dried at 25°C under vacuum.

16.9 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type IV) were obtained, with a content of 98.3% and a water content of 9.9%.

By drying it at 65°C under vacuum, the water content of this product could be reduced to 5.5% without a different crystalline modification being obtained in the course of this procedure.

Table 1: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I)

```
Diffractometer : Transmission
Monochromator  : Curved Ge(111)
Wavelength     : 1.540598  Cu
Detector       : Linear PSD
Scan Mode      : Debye-Scherrer / Moving PSD / Fixed omega
2Theta scan

! Peak search parameters : Expected halfwidth :  .150
!                         Significance level :  2.5
!                         Peak height level  :   10

Peaklist [ Range 1 : 2Theta = 5.000 34.980 .020  Imax = 765 ]

!      D        2Theta    I(rel)    I(abs)    FWHM      h    k    l

  13.474630     6.5544    100.0       755    .2200
   8.979750     9.8420     18.5       140    .1600
   6.936035    12.7526     20.3       153    .1600
   6.662427    13.2786     38.3       289    .0800
   6.497896    13.6164     29.4       222    .1200
   6.323596    13.9935     18.8       142    .0200
   6.148863    14.3933     14.0       106    .0400
   5.966675    14.8352     15.5       117    .1200
   5.593548    15.8309     27.5       208    .2200
   5.368022    16.5006     19.7       149    .1127
   5.282104    16.7709     42.5       321    .2000
   4.977751    17.8044     23.6       178    .1800
   4.672452    18.9782     32.7       247    .2800
   4.411916    20.1102     34.8       263    .0800
   4.257688    20.8467     34.2       258    .2600
   3.761157    23.6360     13.3       100    .0400
   3.699455    24.0361     22.3       168    .1400
   3.558431    25.0037     14.8       112    .1000
   3.439070    25.8864     21.0       159    .1400
   3.272550    27.2283     22.1       167    .2800
   3.218939    27.6907     17.0       129    .1400
   3.140884    28.3931     17.2       130    .0800
   3.013536    29.6198     13.9       105    .1000
   2.873482    31.0991     15.1       114    .0200
   2.782802    32.1395     16.6       125    .0200
   2.754830    32.4748     20.2       152    .0600
   2.713309    32.9858     15.4       116    .1127
```

Table 2: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type II)

```
Diffractometer : Transmission
Monochromator  : Curved Ge(111)
Wavelength     : 1.540598  Cu
Detector       : Linear PSD
Scan Mode      : Debye-Scherrer / Moving PSD / Fixed omega
2Theta scan

! Peak search parameters : Expected halfwidth :  .150
!                         Significance level :  2.5
!                         Peak height level  :   10

Peaklist [ Range 1 : 2Theta = 5.000 34.980 .020  Imax = 526 ]

!      D        2Theta   I(rel)   I(abs)   FWHM     h   k   l

  12.720530     6.9434   100.0      517   .2600
   8.508053    10.3891    29.4      152   .2400
   6.631466    13.3409    19.6      101   .1200
   5.883504    15.0461    71.2      368   .2200
   5.580025    15.8696    27.8      144   .0800
   5.010988    17.6854    42.5      220   .1400
   4.730443    18.7434    53.6      277   .1400
   4.215807    21.0561    35.5      184   .0400
   3.943879    22.5263    38.8      201   .3600
   3.581969    24.8368    24.8      128   .0200
   3.493985    25.4726    29.6      153   .0400
   3.309171    26.9212    22.7      117   .0200
```

Table 3: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type III)

```
Diffractometer : Transmission
Monochromator  : Curved Ge(111)
Wavelength     : 1.540598  Cu
Detector       : Linear PSD
Scan Mode      : Debye-Scherrer / Moving PSD / Fixed omega
2Theta scan

! Peak search parameters : Expected halfwidth :   .150
!                         Significance level :   2.5
!                         Peak height level  :    10

Peaklist [ Range 1 : 2Theta = 5.000 34.980 .020  Imax = 817 ]

!      D        2Theta   I(rel)   I(abs)   FWHM     h   k   l

  12.933490     6.8289   100.0      786   .1200
  11.036740     8.0043    18.9      149   .0400
   9.945525     8.8842    18.4      145   .1000
   8.877709     9.9554    12.4       98   .0796
   8.640580    10.2293    49.6      390   .1000
   7.873330    11.2292     6.4       50   .1000
   7.144004    12.3799     7.6       59   .0800
   6.948557    12.7295    20.3      159   .1000
   6.659956    13.2835    10.1       80   .0400
   6.466239    13.6834     7.6       60   .0200
   6.305060    14.0349    37.6      296   .1000
   6.154434    14.3802    16.4      129   .0400
   6.057193    14.6123    15.3      121   .0600
   5.920458    14.9517    17.6      139   .1000
   5.738533    15.4285    48.9      385   .1000
   5.530167    16.0136    30.3      238   .1000
   5.322477    16.6428    18.1      143   .0600
   5.245302    16.8894    47.4      372   .0800
   5.154604    17.1888    20.9      164   .0796
   5.038273    17.5888    30.8      242   .1000
   4.980502    17.7945    10.7       84   .0796
   4.759336    18.6286    31.6      248   .1200
   4.702846    18.8544    24.3      191   .0796
   4.575841    19.3827    15.6      122   .0800
   4.478961    19.8061    25.9      204   .1000
   4.377158    20.2716    48.1      378   .1000
   4.309006    20.5957    11.9       93   .0796
   4.242777    20.9207    31.3      246   .0800
   4.051441    21.9207    10.3       81   .0200
   3.940356    22.5467    67.8      533   .1200
   3.782452    23.5010    12.4       98   .0400
   3.609291    24.6458     9.5       75   .0200
   3.523157    25.2582    27.0      212   .2000
   3.460874    25.7205    43.4      341   .0800
   3.408545    26.1223    12.4       98   .0796
   3.341048    26.6596    16.1      127   .2000
   3.273575    27.2196    28.4      223   .1400
   3.188038    27.9645    12.6       99   .0200
   3.160110    28.2168    12.5       98   .0400
   3.103472    28.7427    15.0      118   .0800
   3.052658    29.2317    13.9      109   .0600
   3.017419    29.5808    27.7      218   .1400
   2.970195    30.0621    10.6       83   .1200
   2.921067    30.5800    13.9      109   .0200
   2.899222    30.8161     9.6       76   .0796
   2.870572    31.1314     9.6       75   .0400
   2.830661    31.5817    11.0       86   .0200
   2.758126    32.4349    11.3       89   .0400
   2.733265    32.7382    13.2      104   .0600
   2.695836    33.2058    13.7      108   .0800
   2.660160    33.6643    11.7       92   .1000
   2.609572    34.3369     9.2       72   .0200
```

Table 4: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type IV)

```
Diffractometer : Transmission
Monochromator  : Curved Ge(111)
Wavelength     : 1.540598  Cu
Detector       : Linear PSD
Scan Mode      : Debye-Scherrer / Moving PSD / Fixed omega
2Theta scan

! Peak search parameters : Expected halfwidth :   .150
!                         Significance level :   2.5
!                         Peak height level  :    10

Peaklist [ Range 1 : 2Theta = 5.000 34.980 .020  Imax = 473 ]

!      D        2Theta    I(rel)    I(abs)    FWHM      h    k    l

  13.398610     6.5916     97.7       446    .1600
  12.930100     6.8307    100.0       457    .0915
  11.033220     8.0069     19.2        88    .0800
   9.952926     8.8776     16.7        76    .1200
   8.912272     9.9167     25.5       116    .1600
   8.626970    10.2455     48.9       223    .0800
   6.931997    12.7600     37.4       171    .1000
   6.651761    13.3000     39.7       181    .1200
   6.499623    13.6127     32.8       150    .0800
   6.309299    14.0254     47.0       215    .1600
   6.161306    14.3641     25.1       115    .1200
   5.917463    14.9593     27.0       124    .1000
   5.736254    15.4347     49.8       227    .0800
   5.544314    15.9724     36.7       168    .1600
   5.255854    16.8553     62.1       284    .2400
   5.172075    17.1303     29.5       135    .0915
   5.035719    17.5978     37.0       169    .1200
   4.978813    17.8006     31.3       143    .0400
   4.758441    18.6321     40.7       186    .1000
   4.688853    18.9112     46.0       210    .0915
   4.577465    19.3757     29.5       135    .0915
   4.479376    19.8043     35.5       162    .1000
   4.383704    20.2410     63.6       290    .1200
   4.246196    20.9037     59.5       272    .1400
   4.088125    21.7216     19.7        90    .0200
   3.941748    22.5386     62.9       288    .1400
   3.778991    23.5229     27.9       128    .0400
   3.696576    24.0551     30.5       139    .1000
   3.523769    25.2537     35.6       163    .2400
   3.459683    25.7295     44.7       204    .0800
   3.338511    26.6803     28.7       131    .0200
   3.273450    27.2206     45.5       208    .1000
   3.135320    28.4446     23.6       108    .0600
   3.108154    28.6985     25.9       118    .0200
   3.018687    29.5681     34.4       157    .1400
   2.923031    30.5589     21.9       100    .0200
   2.844431    31.4249     18.4        84    .0200
   2.749393    32.5408     28.5       130    .1200
   2.713739    32.9804     25.6       117    .0200
   2.663207    33.6246     19.6        90    .0600
   2.613490    34.2838     17.4        80    .0200
```

CLAIMS:

1. A crystalline salt of 5-methyl-(6R,S)-, -(6S)- or -(6R)-tetrahydrofolic acid said crystalline salt having a water of crystallisation of at least one equivalent per equivalent of 5-methyltetrahydrofolic acid.

2. A crystalline salt according to claim 1, of 5-methyl-(6S)- or -(6R)-tetrahydrofolic acid.

3. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)- and -(6R)-tetrahydrofolic acid having $\geqq$ 3 equivalents of water.

4. A crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I) said crystalline salt having a water of crystallisation of at least one equivalent per equivalent of 5-methyltetrahydrofolic acid.

5. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 5.3, 6.9, 18.7 and 21.1 (Type II).

6. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.8, 10.2, 15.4 and 22.5 (Type III).

7. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.6, 15.9, 20.2 and 22.5 (Type IV).

8. A method of producing crystalline salts of 5-methyl-(6R,S)-, -(6S)- and 5-methyl-(6R)-tetrahydrofolic acid, comprising subjecting a salt of 5-methyl-(6R,S)-, -(6S)- or -(6R)-tetrahydrofolic acid in a polar medium to a thermal treatment, at a temperature above 60° C., and thereafter crystallising said salt from the resultant heated solution.

9. A method according to claim 8, wherein the crystallisation is effected after thermal treatment at a temperature above 85° C.

10. A method according to claim 8, wherein the crystallisation is effected from a solution.

11. A method according to claim 8, wherein the crystallisation is effected from a suspension.

12. A method according to claim 10, characterised in that crystallisation is effected from water or from a mixture of water and an organic solvent which is miscible with water.

13. A method according to claim 8, wherein said salt is an alkaline earth salt.

14. A method according to claim 8, wherein said salt is calcium.

15. A method of producing 5-methyl-(6S)-tetrahydrofolic acids with 2 theta values of 5.3, 6.9, 18.7 and 21.1 (Type II) comprising drying sufficiently 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

16. A method of producing 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.8, 10.2, 15.4 and 22.5 (Type III) comprising subjecting to sufficient thermal treatment at above 90° C., a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

17. A method of producing 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.6, 15.9, 20.2, 22.5 (Type IV) comprising subjecting to sufficient thermal treatment at above 95° C., a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

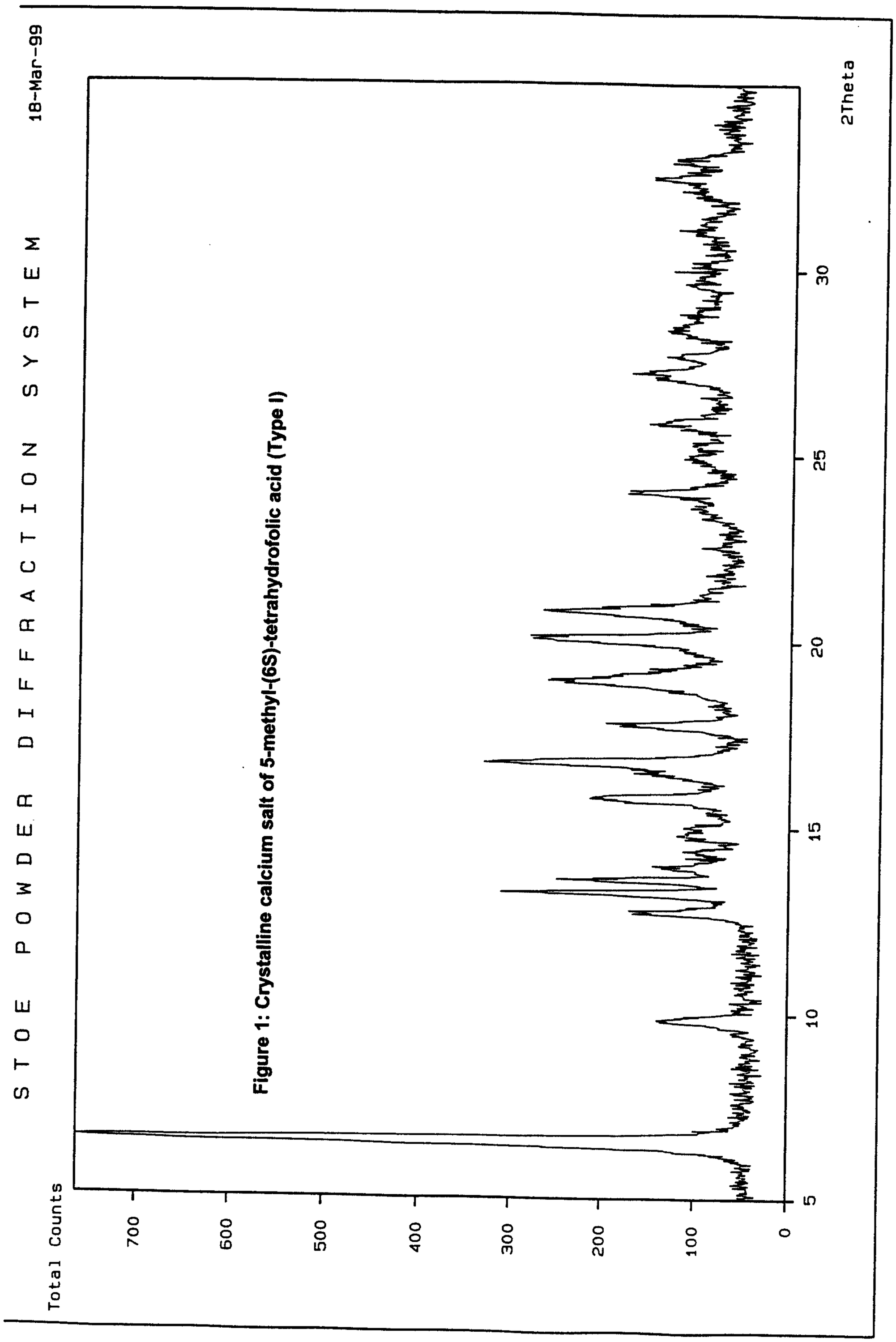


Figure 1: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I)

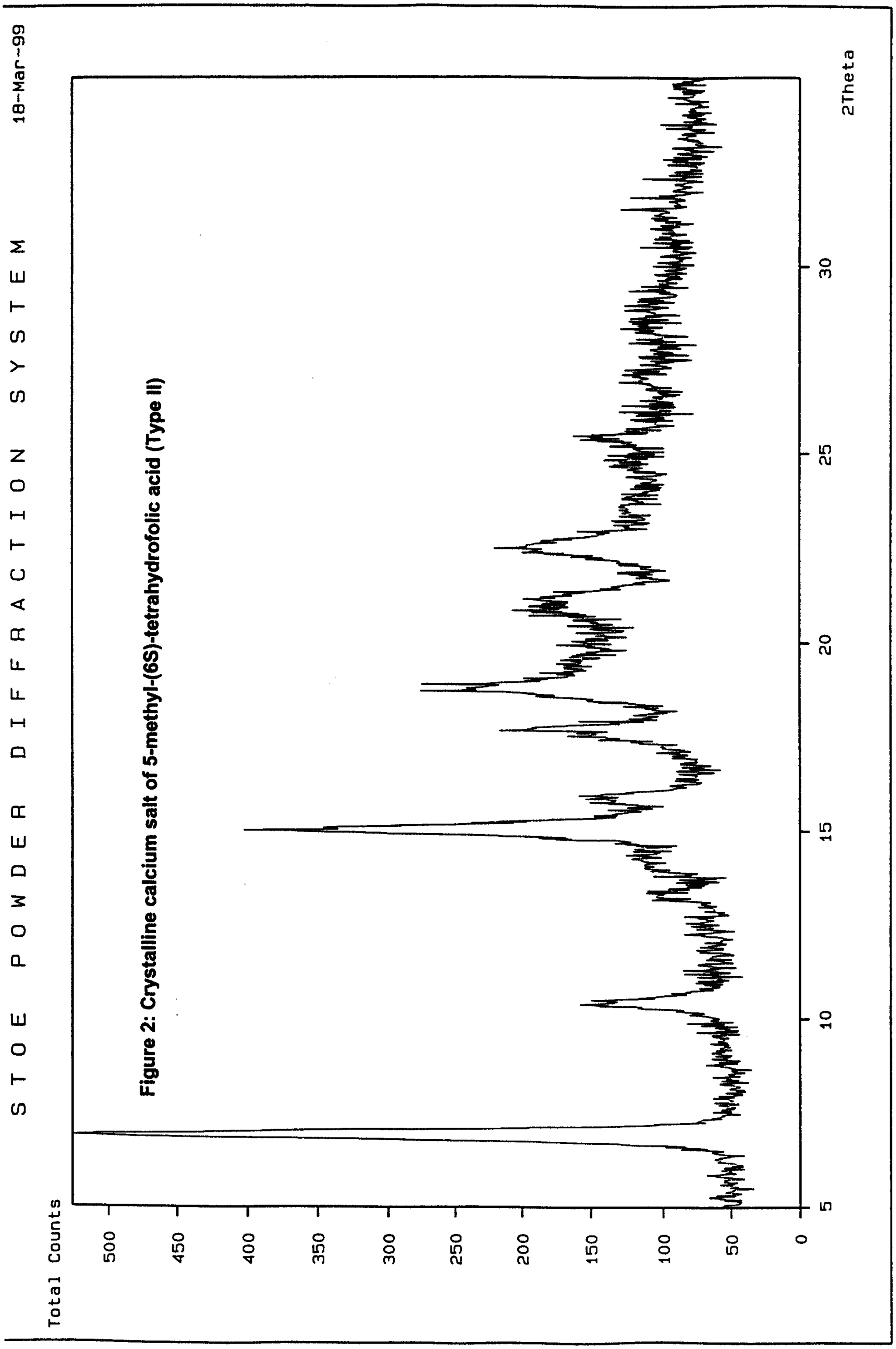


Figure 2: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type II)

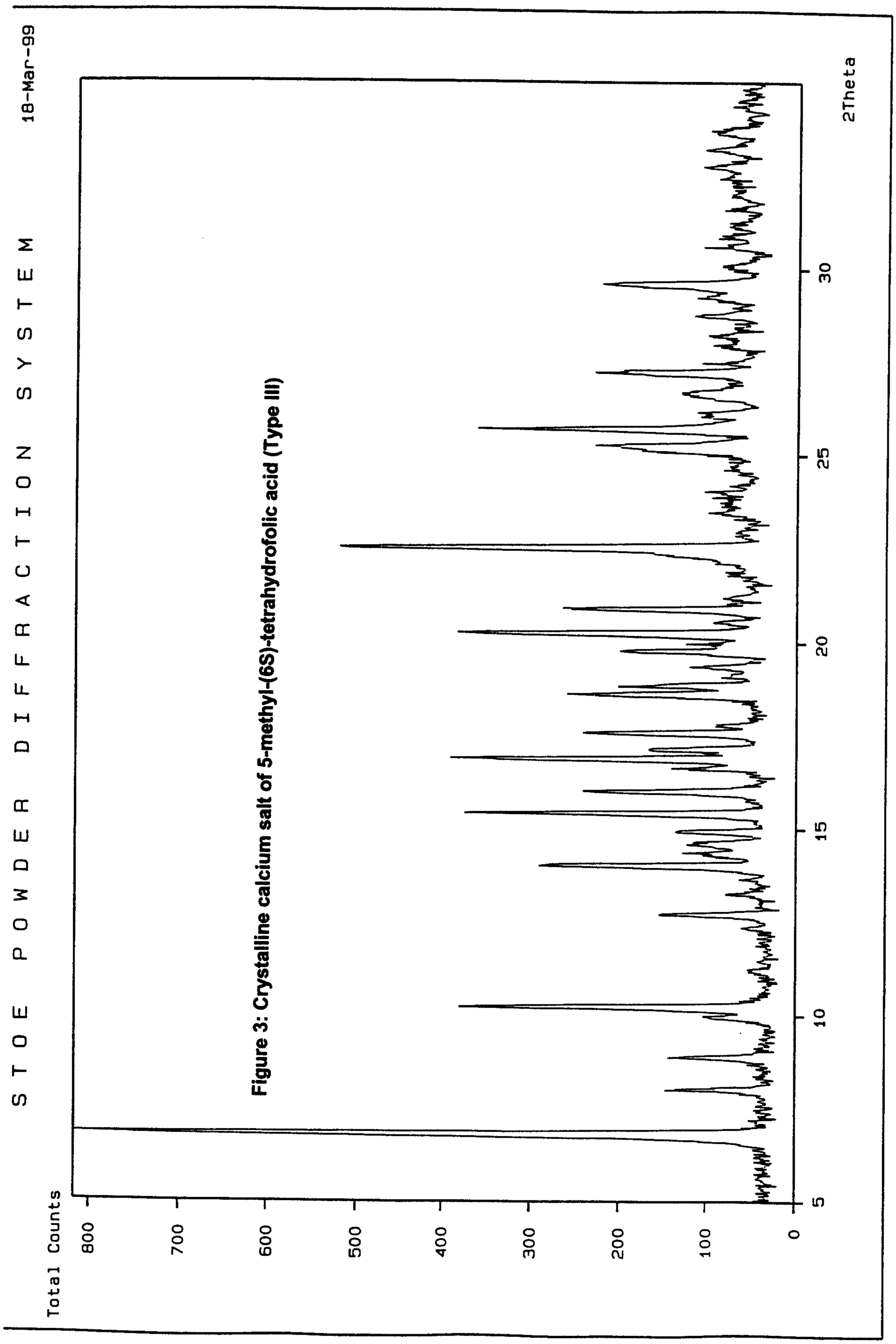


Figure 3: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type III)

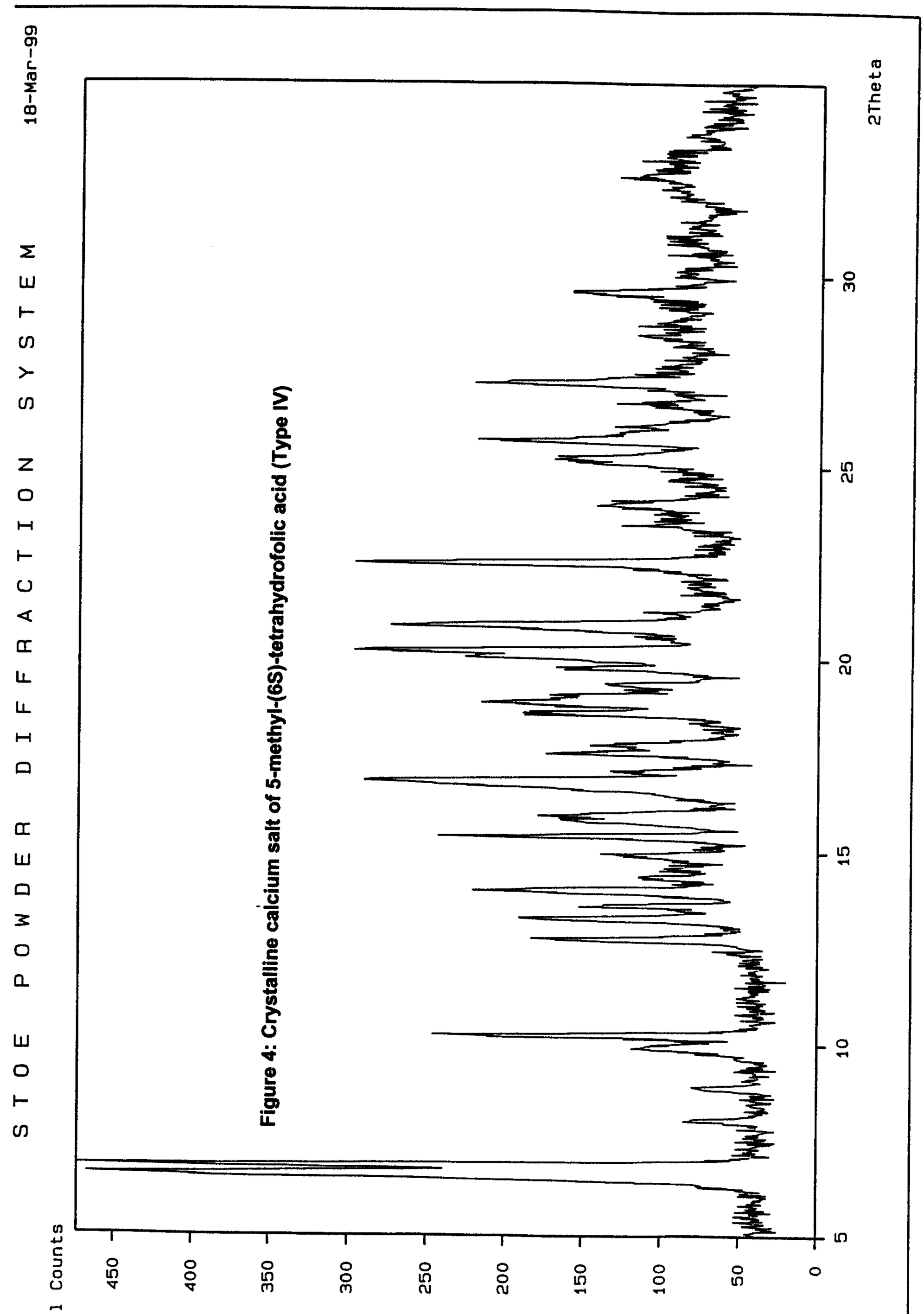
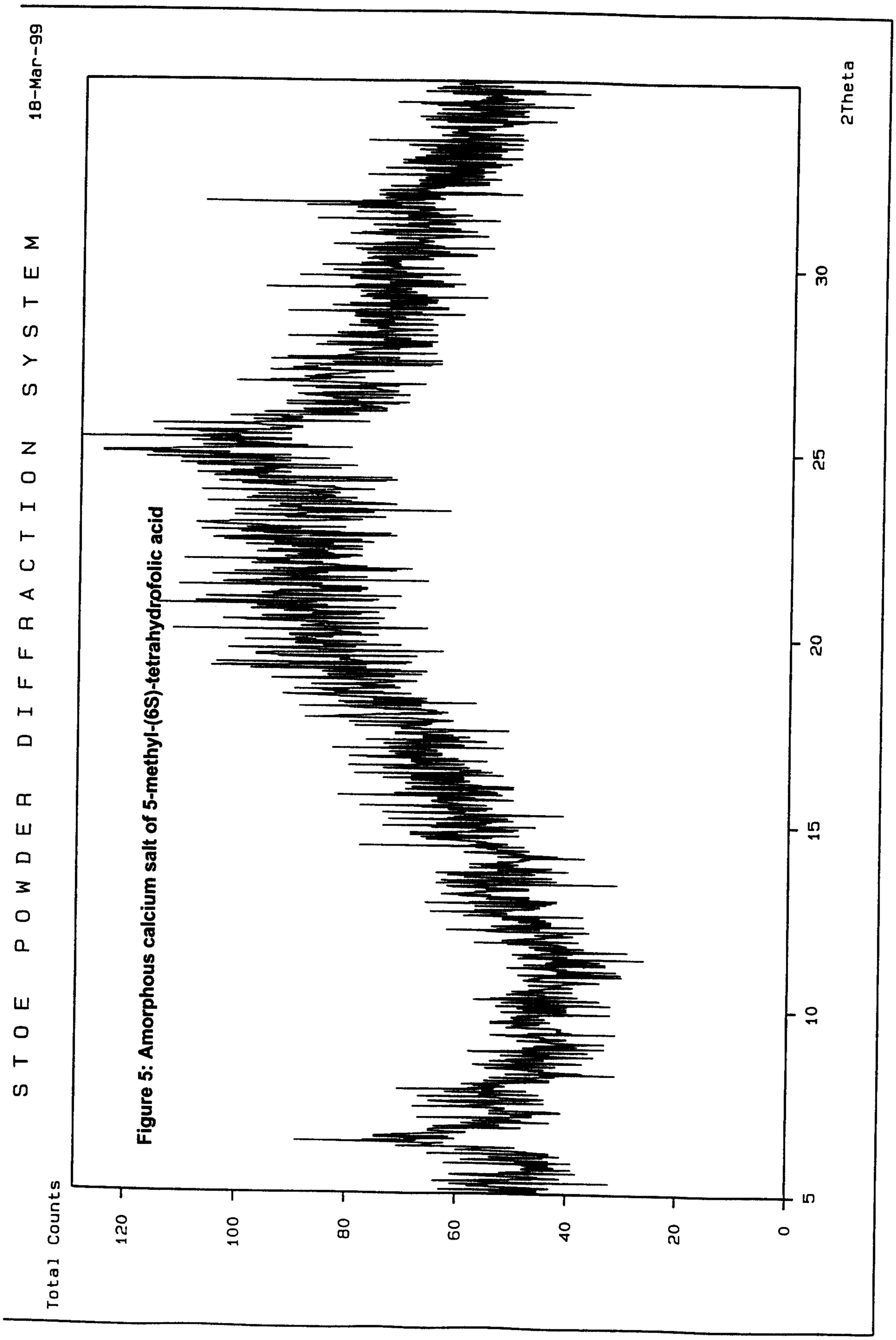


Figure 4: Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type IV)


STOE POWDER DIFFRACTION SYSTEM
18-Mar-99
Total Counts
2Theta
120
100
80
60
40
20
0
5
10
15
20
25
30
Figure 5: Amorphous calcium salt of 5-methyl-(6S)-tetrahydrofolic acid

STOE POWDER DIFFRACTION SYSTEM