(19) DANMARK

(10) DK/EP 3066111 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 07 K 5/02 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2019-08-05**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2019-06-05**

(86) Europæisk ansøgning nr.: **14803064.6**

(86) Europæisk indleveringsdag: **2014-11-06**

(87) Den europæiske ansøgnings publiceringsdag: **2016-09-14**

(86) International ansøgning nr.: **EP2014073957**

(87) Internationalt publikationsnr.: **WO2015067708**

(30) Prioritet: **2013-11-08 IT MI20131856**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Gnosis S.p.A., Piazza Filippo Meda 3, 20121 Milano, Italien**

(72) Opfinder: **BIANCHI, Davide, c/o GNOSIS S.p.A., Via Lavoratori Autobianchi, 1, 20033 Desio (MI), Italien**
VALETTI, Marco, c/o Gnosis S.p.A., Via Lavoratori Autobianchi 1, 20033 Desio (MI), Italien
BAZZA, Paola, c/o Gnosis S.p.A., Via Lavoratori Autobianchi 1, 20033 Desio (MI), Italien

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **Krystallinske former af S-acetyl-glutathion, fremstilling og anvendelser deraf i farmaceutiske og nutraceutiske formuleringer**

(56) Fremdragne publikationer:
US-A- 2 760 956
US-A1- 2009 136 993
CAIRA M R: "CRYSTALLINE POLYMORPHISM OF ORGANIC COMPOUNDS", TOPICS IN CURRENT CHEMISTRY, SPRINGER, BERLIN, DE, vol. 198, 1 January 1998 (1998-01-01), pages 163-208, XP001156954, ISSN: 0340-1022, DOI: 10.1007/3-540-69178-2_5

DESCRIPTION

Field of invention

[0001] The present invention relates to novel crystalline form A of S-acetyl glutathione (SAG) which is useful in the preparation of pharmaceutical or nutraceutical compositions.

Background to the invention

[0002] Glutathione (GSH) is a compound which, in its reduced form, constitutes an excellent antioxidant and therefore a defence against the damage caused by free radicals to higher organisms. S-acetyl-glutathione (SAG) (see fig. 1) is a synthetic derivative thereof which protects GSH against oxidation, and simultaneously releases it easily by hydrolysis.

[0003] GSH or γ-L-glutamyl-L-cysteinylglycine is a tripeptide consisting of glutamic acid, cysteine and glycine, characterised by an atypical peptide bond, namely the bond that binds the nitrogen of cysteine to the carboxyl in γ glutamic acid. It is the main thiol compound with the lowest molecular weight present in both animal and plant cells (about 95% of the total). Its function is to maintain in the reduced state the -SH groups of many enzymes and proteins whose oxidation (with the formation of S-S intra- and intermolecular disulphide bridges) leads, in most cases, to inactivation or loss of the biological function of the enzyme or protein.

[0004] GSH is considered to be one of the most important intracellular antioxidants produced naturally by the human body. However, chronic oxidative stress reduces the cell levels of GSH, and it is often appropriate to replenish its levels with the aid of diet supplements.

[0005] It is commonly believed that the GSH intake obtained from the diet or with the use of diet supplements is easily used by the issues, but in reality it is not absorbed "as is", but hydrolysed into its three constituent amino acids by a gamma-glutamyl transpeptidase present in the intestine. After being absorbed and introduced into the bloodstream, said amino acids are distributed to the various tissues wherein they implement the pool of amino acids with which the body cells synthesise endogenous GSH. It is therefore necessary to use a high oral dose in order to guarantee significant absorption. When Witschi et al. evaluated the increase in the blood levels of glutathione, cysteine and glutamate after oral administration of GSH to seven healthy volunteers, no significant increases were observed at doses of up to 3 g per dose (Witschi A et al., J. Clin. Pharmacol. 43 (6), 667 - 1992).

[0006] Sublingual administration, which guarantees better bioavailability, can be used as an alternative to oral administration of GSH.

[0007] Finally, in the pharmaceutical field, prophylaxis based on GSH is used in some cases by

parenteral, intramuscular or slow intravenous administration, for example as prophylaxis for neuropathy resulting from chemotherapy with cisplatin or analogues.

[0008] The use of SAG as a precursor is a good alternative to replenish the reduced GSH levels in the body. In fact, acetylation of the sulphur atom prevents the decomposition of GSH and facilitates its absorption through the intestinal wall, thus enabling the molecule to pass extensively into the cells.

[0009] The SAG thus assimilated by the tissues is hydrolysed by cytoplasmic thioesterase and, by hydrolysis of the acetyl group, produces reduced GSH which is available for all the biological functions wherein it is required.

[0010] The addition of SAG to cultures of fibroblasts originating from individuals suffering from a genetic glutathione synthetase deficiency has proved able to replenish the intracellular level of GSH effectively (Okun JG et al., J. Inherit. Metab. Dis. 27(6), 783 - 2004). SAG is also more stable in the plasma and has proved more effective than GSH in replenishing the cell levels of GSH impoverished by viral infections (Vogel JU et al., Med. Microbiol. Immunol. 194, 55 - 2005) (Fraternale A et al., Antiviral Res. 77, 120 - 2008). Finally, SAG exhibits an interesting non-GSH-dependent activity that induces apoptosis in some human tumour cell lines *in vitro.* (Locigno R et al., Int. J. Oncol. 20, 69 - 2002).

[0011] Identification and characterisation of the polymorphic forms, and of the experimental conditions for obtaining them, are very important parameters for a compound designed for nutraceutical and/or pharmaceutical use.

[0012] The synthesis of SAG has already been claimed in a Japanese patent (see Chemical Abstract 97-7222755s) and in WO92/00320. However, the authors only disclose a general method for obtaining it, without investigating the existence of polymorphic forms in any way.

[0013] As stated above, a number of difficulties are involved in the absorption of GSH, which are partly solved by the use of the SAG derivative. However, the absorption of said compound may be adversely affected by the existence of polymorphic forms thereof having different physicochemical characteristics that influence its dissolution rate, solubility and therefore bioavailability, not to mention the different behaviour of the powders during the preparation of the various formulations.

[0014] No experimental condition or preliminary indication for crystallisation and drying that suggests the existence of polymorphic forms of SAG has ever been disclosed in any patent or patent application.

Description of figures

[0015]

FIGURE 1: glutathione (GSH) and S-acetyl glutathione (SAG) structures

FIGURE 2: 1H-NMR spectrum of SAG form A

FIGURE 2A: expansion of the 1H-NMR spectrum of SAG form A in the 1.8-5 ppm range

FIGURE 3: 1H-NMR spectrum of SAG form B (not part of the invention)

FIGURE 3A: expansion of the 1H-NMR spectrum of SAG form B in the 1.8-5 ppm range (not part of the invention)

FIGURE 4: XRD diffractogram of SAG form A

FIGURE 5: XRD diffractogram of SAG form B (not part of the invention)

FIGURE 6: FTIR spectrum of SAG form A

FIGURE 7: FTIR spectrum of SAG form B (not part of the invention)

FIGURE 8: thermogravimetric analysis (TGA) of SAG form A

FIGURE 9: thermogravimetric analysis (TGA) of SAG form B (not part of the invention)

FIGURE 10: DSC thermogram of SAG form A

FIGURE 11: DSC thermogram of SAG form B (not part of the invention)

FIGURE 12: DSC cooling thermogram of SAG form B (not part of the invention)

Description of the invention

[0016] We have now surprisingly found that SAG exists not only in the amorphous form, but also in at least two polymorphic forms characterised by different physicochemical properties, which are useful as ingredients of pharmaceutical or nutraceutical compositions.

[0017] The experimental evidence for the existence of said two polymorphic forms, described in the present invention as form A and form B, wherein form B is not part of the invention, is supplied by the analyses described below, conducted both in solution and in the solid state.

[0018] The samples of the two crystalline forms subjected to 1H-NMR analysis (Figures 2, 2A, 3 and 3A) produced a spectrum highly consistent with the chemical formula of the stated compound, indicating that there is no spectral difference between them in solution.

[0019] Conversely, the analyses performed directly on the substance in the solid state clearly demonstrate the presence of polymorphic forms.

[0020] XRD: the analyses conducted with the X-ray diffractometer indicated significant differences in the crystallographic properties of the two samples (Figures 4 and 5). The number and intensity of no less than 20 diffraction peaks clearly indicate that they possess two different cell types, and therefore that there are two distinct crystalline forms of the same chemical compound.

[0021] Tables 1 and 2 show the best-resolved diffraction peaks, relating to polymorphic forms A and B respectively. The values shown in bold type correspond to the peaks characterising the two forms.

Table 1

2 theta [°]	d-value	I/I_0
5.2	17.1113	63
10.3	8.6143	55
15.4	5.7636	76
18.6	4.7765	100
19.7	4.4981	81
20.4	4.3496	71
21.1	4.2148	87
25.1	3.5420	70
25.7	3.4607	76
27.0	3.3043	57
27.6	3.2337	76
27.9	3.1928	98
32.7	2.7346	77
35.3	2.5376	66
36.3	2.4753	84

Table 2

2 theta [°]	d-value	I/Io
4.2	21.2221	20
12.7	6.9861	22
13.0	6.8251	21
14.9	5.9405	16
17.3	5.1214	29
17.7	5.0122	33
21.0	4.2227	100
21.3	4.1717	98
21.9	4.0513	45

2 theta [°]	d-value	I/Io
22.5	3.9413	43
24.7	3.5956	40
25.1	3.5476	59
30.2	2.9568	58
32.6	2.7477	44

[0022] IR: here again, the spectra recorded by FTIR on the substances in the solid state (Figures 6 and 7) exhibited different spectral bands, clearly indicating the presence of two different crystalline forms. Form A presents *(inter alia)* a characteristic NH stretching band at 3344 cm^{-1} and characteristic carbonyl stretching bands at 1726, 1687 and 1663 cm^{-1}. Form B presents *(inter alia)* characteristic NH stretching bands at 3370 and 3355 cm^{-1} and characteristic carbonyl stretching bands at 1701, 1677 and 1648 cm^{-1}.

[0023] Although the 1H-NMR spectra excluded the presence of solvents, the samples were also subjected to thermal analyses, namely TGA and DSC.

[0024] TGA: thermogravimetric analyses, conducted on two samples, categorically exclude the presence of "solvates" and indicate a marked weight loss, due to decomposition, at a temperature much higher than 150°C (Figures 8 and 9).

[0025] DSC: The thermograms confirm decomposition at around 200°C in both polymorphs, and although the endothermic peak, at 208.2°C for form A and 191.4°C for form B, presents a fairly clear start which could misleadingly indicate a fusion, it relates to decomposition with weight loss (Figures 10 and 11).

[0026] However, the two thermograms differ in terms of the presence, in polymorph B, of a weak endothermic event at about 135°C. Said event, which is perfectly reversible, can be seen in the cooling thermogram of the compound, as an analogous exothermic event at a slightly lower temperature (Figure 12).

[0027] On the basis of these data, it can therefore be concluded that SAG exists in at least two different polymorphic forms, A and B, characterised by different physicochemical properties.

[0028] One object of the present invention is therefore a crystalline form of SAG called form A, characterised by an X-ray powder diffraction spectrum, obtained with α_1 (λ =1.54060Å) and α_2 (λ = 1.54439Å) copper radiation, as shown in Figure 4, and having characteristic peaks, expressed in degrees 2-theta [°], at 5.2, 10.3, 15.4, 18.6, 19.7, 35.3, 36.3 ± 0.2.

[0029] In the XRD diffractogram, an additional group of characterising diffraction peaks,

expressed in degrees 2-theta [°], is represented by those at 20.4, 21.1, 25.1, 25.7, 27.0, 27.6, 27.9, 32.7 ± 0.2.

[0030] Crystalline form A is characterised by an IR spectrum, obtained with a potassium bromide matrix, as shown in Figure 6, having characteristic absorption bands at 3344, 1726, 1687 and 1663 cm^{-1} (*inter alia).*

[0031] Crystalline form A is also characterised by a DSC pattern, obtained with a heating rate of 10.00°C/min, having an endothermic peak between 190°C and 210°C, connected to the decomposition of the compound, followed by other disorderly endothermic events, as shown in Figure 10.

[0032] The crystalline form of SAG called form B, which is not part of the invention, is characterised by an X-ray powder diffraction spectrum, obtained with α_1 (λ =1.54060Å) and α_2 (λ =1.54439Å) copper radiation, as shown in Figure 5 and having characteristic peaks, expressed in degrees 2-theta [°], at 4.2, 12.7, 13.0, 17.3, 17.7, 30.2 ± 0.2. In the XRD diffractogram, an additional group of characterising diffraction peaks, expressed in degrees 2-theta [°], is represented by those at 14.9, 21.0, 21.3, 21.9, 22.5, 24.7, 25.1, 32.6 ± 0.2.

[0033] Crystalline form B is characterised by an IR spectrum, obtained with a potassium bromide matrix, as shown in Figure 7, having characteristic absorption bands at 3370, 3355, 1701, 1677 and 1648 cm^{-1} (*inter alia)*.

[0034] Crystalline form B is also characterised by a DSC pattern, obtained with a heating rate of 10.00°C/min, having an endothermic decomposition peak between 180°C and 200°C, connected to the decomposition of the compound, followed by other disorderly endothermic events, and a characteristic endothermic peak at about 135°C, as shown in Figure 11.

[0035] A further object of the present invention is a method for the production of crystalline forms A of SAG with high yields and chemical purity.

[0036] Crystalline forms A and B are obtainable by crystallising SAG with mixtures of solvents such as water-acetone, water-ethanol and water-methanol, preferably water-acetone.

[0037] The most surprising finding, which in particular is not easily deducible even by the skilled person, is that all the mixtures of said solvents are able to provide both polymorph A and polymorph B, and that the discriminating factor is the conditions wherein crystallisation is triggered.

[0038] In fact, the addition of the precipitation solvent (non-solvent) before crystallisation is triggered by water gives rise to polymorphic form B, whereas if crystallisation is triggered by water alone and the non-solvent is only added to increase the yields (complete the precipitation), polymorphic form A is obtained. This behaviour is confirmed by the precipitation

of both polymorphs A and B if the triggering of the crystallisation from water is allowed and the solvent (non-solvent) is added before precipitation of the product is complete.

[0039] Crystalline form A of SAG can be prepared by a process comprising the following steps:

1. a) dissolution of SAG in water at a temperature ranging between 75°C and 80°C;
2. b) immediate cooling of the solution obtained in step a) to a temperature of below 55°C, preferably to a temperature ranging between 45°C and 55°C, followed by further cooling until incipient crystallisation;
3. c) cooling to 20-25°C of the mass obtained in step b) in the presence of minimal stirring (60-120 rpm), followed by continued stirring of the mass at 20-25°C for between 2 and 12 hours;
4. d) slow addition to the suspension obtained in c) of a solvent selected from the group containing acetone, ethanol and methanol, preferably acetone, followed by cooling of the resulting suspension to a temperature ranging between 3°C and 7°C;
5. e) isolation of the solid that separates in step d), to give crystalline form A of SAG.

[0040] Crystalline form B of SAG can be prepared by a process comprising the following steps, said process being not part of the invention:

1. a) dissolution of SAG in water at a temperature ranging between 75°C and 80°C;
2. b) immediate cooling of the solution obtained in step a) to a temperature of 55°C, followed by addition of a solvent selected from the group containing acetone, ethanol and methanol, preferably acetone;
3. c) spontaneous cooling to 20-25°C of the mass obtained in step b) in the presence of minimal stirring (60-120 rpm), followed by continued stirring of the mass at 20-25°C for between 2 and 12 hours;
4. d) cooling of the suspension obtained in step c) to a temperature ranging between 3°C and 7°C;
5. e) isolation of the solid that separates in step d), to give crystalline form B of SAG.

[0041] Conversely, the amorphous form, which is not part of the invention, can be obtained by spray-drying of an aqueous solution of the product.

[0042] The two polymorphic forms A and B and the amorphous form present different physicochemical properties, in particular as regards the quality of the product, its stability, its dissolution rate in water, and the density and flowability of the powders.

[0043] ***Quality, assay value and stability of the various forms*** - Crystalline forms A and B differ due to the presence of different quantities of oxidised GSH (GSSG), because the crystallisation of polymorph A gives rise to an increase in GSSG (about 1% more). This GSSG

does not only derive from oxidation of the residual GSH present in the reaction environment, but also of that deriving from hydrolysis of SAG during crystallisation, albeit in minimal quantities. The percentage of GSSG is much higher in the amorphous form due to the drying conditions, which increase hydrolysis and the corresponding oxidation. This does not affect the quality of the product, because GSSG, like SAG, is able to replenish GSH after absorption.

[0044] When samples of polymorphs A and B were subjected to heat and mechanical stresses, the possibility of conversion of one polymorph to the other under the conditions used was not found.

[0045] The stability of the various solid forms was tested as described in the European Pharmacopoeia (EP), by conducting accelerated stability tests at 50°C for 6 months. The results are set out in Table 3 as internal standardisation (% areas of ingredients) and as SAG assay value.

Table 3

Months	Type of solid	Unknown impurities (total)	Unknown impurities (single)	GSH	GSSG	SAG	SAG - assay value
0	Amorphous	1.4%	0.4%	1.0%	2.5%	95.1%	96.5%
1.5	Amorphous	1.9%	0.9%	1.6%	2.5%	94.6%	94.9%
3	Amorphous	2.3%	1.2%	2.4%	2.8%	91.3%	92.4%
4.5	Amorphous	2.7%	1.3%	2.8%	2.9%	90.4%	91.5%
6	Amorphous	3.5%	1.5%	2.5%	3.9%	88.6%	89.9%
Months	Type of solid	Unknown impurities (total)	Unknown impurities (single)	GSH	GSSG	SAG	SAG - assay value
0	Form A	1.0%	0.4%	0.1%	2.2%	96.3%	98.6%
1.5	Form A	1.7%	0.5%	0.1%	2.2%	95.5%	98.3%
3	Form A	1.9%	0.5%	0.2%	2.3%	95.1%	97.8%
4.5	Form A	2.0%	0.7%	0.2%	2.4%	94.7%	97.6%
6	Form A	2.0%	0.9%	0.3%	2.4%	94.4%	97.4%
Months	Type of solid	Unknown impurities (total)	Unknown impurities (single)	GSH	GSSG	SAG	SAG - assay value
0	Form B	0.8%	0.2%	0.2%	1.1%	97.7%	99.3%
1.5	Form B	0.9%	0.5%	0.3%	1.2%	97.1%	99.1%
3	Form B	1.1%	0.7%	0.7%	0.8%	96.7%	98.7%
4.5	Form B	1.3%	0.7%	0.9%	1.1%	96.0%	98.3%
6	Form B	2.0%	0.8%	1.0%	0.9%	95.7%	98.1%

[0046] As will be seen from the data in Table 3, the amorphous form is much less stable than the crystalline forms, and of the latter, polymorphic form B is characterised by a higher purity and assay value.

[0047] ***Dissolution rate*** - Of the two crystalline forms, form B has the most rapid dissolution rate, and is therefore the most suitable for oral formulations, whose dissolution rate influences the absorption rate. Only the amorphous form dissolves more rapidly, but the quality and stability of the product are unsuitable for its use.

[0048] ***Powder density*** - As regards this aspect, study of the two crystalline forms demonstrates that polymorphic form B has a higher density (0.4 g/mL) than form A (0.2-0.25 g/mL). This parameter influences the flowability and compressibility of the powder, and therefore its use for the preparation of solid formulations, especially tablets. The powders of polymorphic form A therefore present better flowability.

[0049] Crystalline forms A and B of SAG can be formulated in pharmaceutical or nutraceutical compositions suitable for oral or parenteral administration, using conventional techniques and excipients.

[0050] A further object of the present invention is therefore pharmaceutical or nutraceutical compositions containing crystalline forms A of SAG.

[0051] A further object is the use of crystalline forms A of SAG for the preparation of medicaments or diet supplements.

[0052] A further object is the use of crystalline forms A of SAG for the preparation of vials containing powdered SAG for injectable parenteral administration.

[0053] The following examples further illustrate the invention.

EXAMPLES

[0054] The XRD spectra were obtained with a RIGAKU-MINIFLEX diffractometer. The radiations used were α_1 and α_2 (λ = 1.54060Å and λ =1.54439Å, respectively) copper radiation.

[0055] The FTIR spectra were obtained with a Perkin-Elmer FTIR Spectrum-one instrument. The samples were analysed as KBr tablets without vacuum, with a 1:100 dilution.

[0056] The TGA patterns were obtained with a Universal V2.6D TA instrument. The temperature range explored was 0°C → 300°C, with a scanning rate of 10°C/min.

[0057] The DSC thermograms were obtained with a Perkin Elmer DSC6 instrument. The

temperature range explored was 30°C → 350°C, with a scanning rate of 10°C/min. In the case of Figure 12, the DSC thermogram was obtained by heating from 30°C to 145°C at the rate of 10°C/min, the sample then being maintained for 5 min at 145°C and finally cooled from 145°C to 30°C at the rate of 10°C/min.

[0058] The 1H-NMR spectra were obtained with a Varian Gemini 200 instrument operating at 200 MHz, using D_2O as solvent.

Example

Preparation of SAG in crystalline form A

[0059] 5 g of crude SAG is placed under stirring and heated to 75°C in 40 mL of demineralised water. The reaction mass is heated to 75°C-80°C. After dissolution, the solution is immediately cooled to a temperature of under 55°C, preferably between 45 and 55°C. Cooling continues until crystallisation begins. Stirring is minimised and the solution is cooled to 20-25°C, at which temperature it is left under stirring for 2-12 h until precipitation is complete. Subsequently, again with minimal stirring, 40 mL of acetone is added in about 30-50 min. The addition is slow to prevent the formation of even a few crystals of polymorph B. The resulting suspension is then brought to 5°C ± 2°C and maintained under slow stirring
(60-120 rpm) for about 1 h. At the end of that time the reaction mass is filtered to obtain a white solid, which is washed with anhydrous acetone (2 x 10 mL). 8.4 g of wet solid is thus obtained, which is left to dry at 50°C, 5 mbar of residual vacuum for 14-18 h. 4.3 g (86%) of white crystalline solid corresponding to crystalline form A is obtained after drying.

[0060] The analytical profile of the product thus obtained is:

Assay value: 98.6% (as is)

Impurities: Total: 1.0%; Single known impurities: GSH (0.1%), GSSG (2.2%);

Water 1.4%

Residual acetone: < 500 ppm

Residual acetic acid: 0.4%

Apparent density: 0.15-0.25 g/mL

[0061] The product thus obtained presents the 1H-NMR spectra shown in Figure 2 and Figure 2A, the XRD diffractogram shown in Figure 4, the FTIR spectrum shown in Figure 6, the TGA pattern shown in Figure 8, and the DSC thermogram shown in Figure 10. The best-resolved

diffraction peaks, and their relative intensities, are shown in Table 1.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO9200320A [0012]

Non-patent literature cited in the description

- **WITSCHI A et al.**J. Clin. Pharmacol., vol. 43, 6667-1992 [0005]
- **OKUN JG et al.**J. Inherit. Metab. Dis., vol. 27, 6783-2004 [0010]
- **VOGEL JU et al.**Med. Microbiol. Immunol., vol. 194, 55-2005 [0010]
- **FRATERNALE A et al.**Antiviral Res., vol. 77, 120-2008 [0010]
- **LOCIGNO R et al.**Int. J. Oncol., vol. 20, 69-2002 [0010]
- CHEMICAL ABSTRACTS, 97-7222755s [0012]

Patentkrav

1. Krystallinsk form, benævnt form A, af S-acetyl-glutathion (SAG), **kendetegnet ved** et røntgenpulverdiffraktionsspektrum, opnået under anvendelse af α_1 og α_2 strålinger af kobber ved henholdsvis 1,54060 Å og 1,54439 Å, med karakteristiske peaks, udtrykt i grader 2-theta [°], ved 5,2, 10,3, 15,4, 18,6, 19,7, 35,3, 36,3 ± 0,2.

2. Krystallinsk form af SAG ifølge krav 1, yderligere **kendetegnet ved** et røntgenpulverdiffraktionsspektrum, som også har karakteristiske peaks, udtrykt i grader 2-theta [°], ved 20,4, 21,1, 25,1, 25,7, 27,0, 27,6, 27,9, 32,7 ± 0,2.

3. Krystallinsk form A af SAG ifølge krav 1 og 2, **kendetegnet ved** et IR-spektrum, opnået i en kaliumbromid-matrix, med karakteristiske absorptionsbånd ved bl.a. 3344, 1726, 1687 og 1663 cm^{-1}.

4. Krystallinsk form A af SAG ifølge krav 1 til 3, **kendetegnet ved** et DSC-diagram med et endotermt dekompositionspeak mellem 190 °C og 210 °C, opnået med en opvarmningshastighed på 10,00 °C/min.

5. Krystallinsk form A af SAG ifølge krav 4, **kendetegnet ved** et DSC-diagram med et endotermt dekompositionspeak ved 208,2 °C.

6. Fremgangsmåde til opnåelse af den krystallinske form A af SAG ifølge krav 1 til 5, omfattende følgende trin:

a) opløsning af SAG i vand ved en temperatur i området fra 75 °C til 80 °C;

b) øjeblikkelig afkøling af opløsningen opnået i trin a) til en temperatur, der er lavere end 55 °C, fortrinsvis til en temperatur i området fra 45 °C til 55 °C, efterfulgt af yderligere afkøling, indtil begyndende krystallisering forekommer;

c) afkøling til 20-25 °C af massen opnået i trin b) under omrøring med en hastighed på 60-120 rpm, efterfulgt af yderligere omrøring af massen ved 20-25 °C i et tidsrum fra 2 til 12 timer;

d) langsom tilsætning til suspensionen opnået i c) af et opløsningsmiddel udvalgt fra gruppen bestående af acetone, ethanol eller methanol, fortrinsvis acetone, efterfulgt af afkøling af den resulterende suspension ved en temperatur i området fra 3 °C til 7 °C;

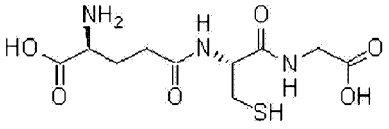
e) genindvinding af det i trin d) præcipiterede faststof, til dannelse af den krystallinske form A af SAG.

7. Nutraceutisk eller farmaceutisk sammensætning indeholdende den krystallinske form A af SAG ifølge krav 1 til 5.

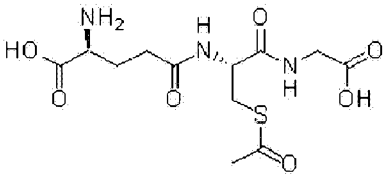
8. Anvendelse af den krystallinske form A af SAG ifølge krav 1 til 5 til fremstilling af en nutraceutisk eller farmaceutisk sammensætning.

DRAWINGS

Figure 1



GSH



SAG

Figure 2

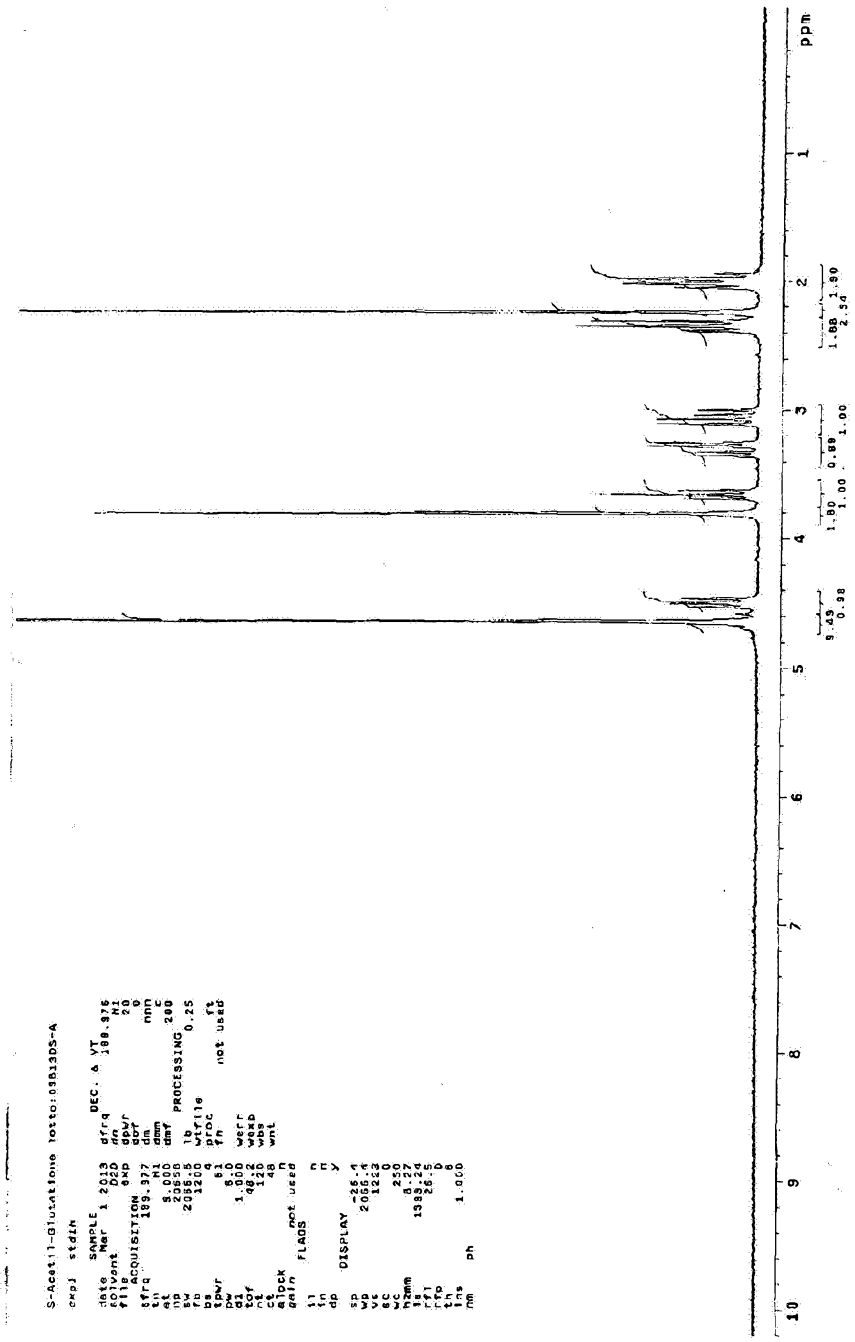


Figure 2A

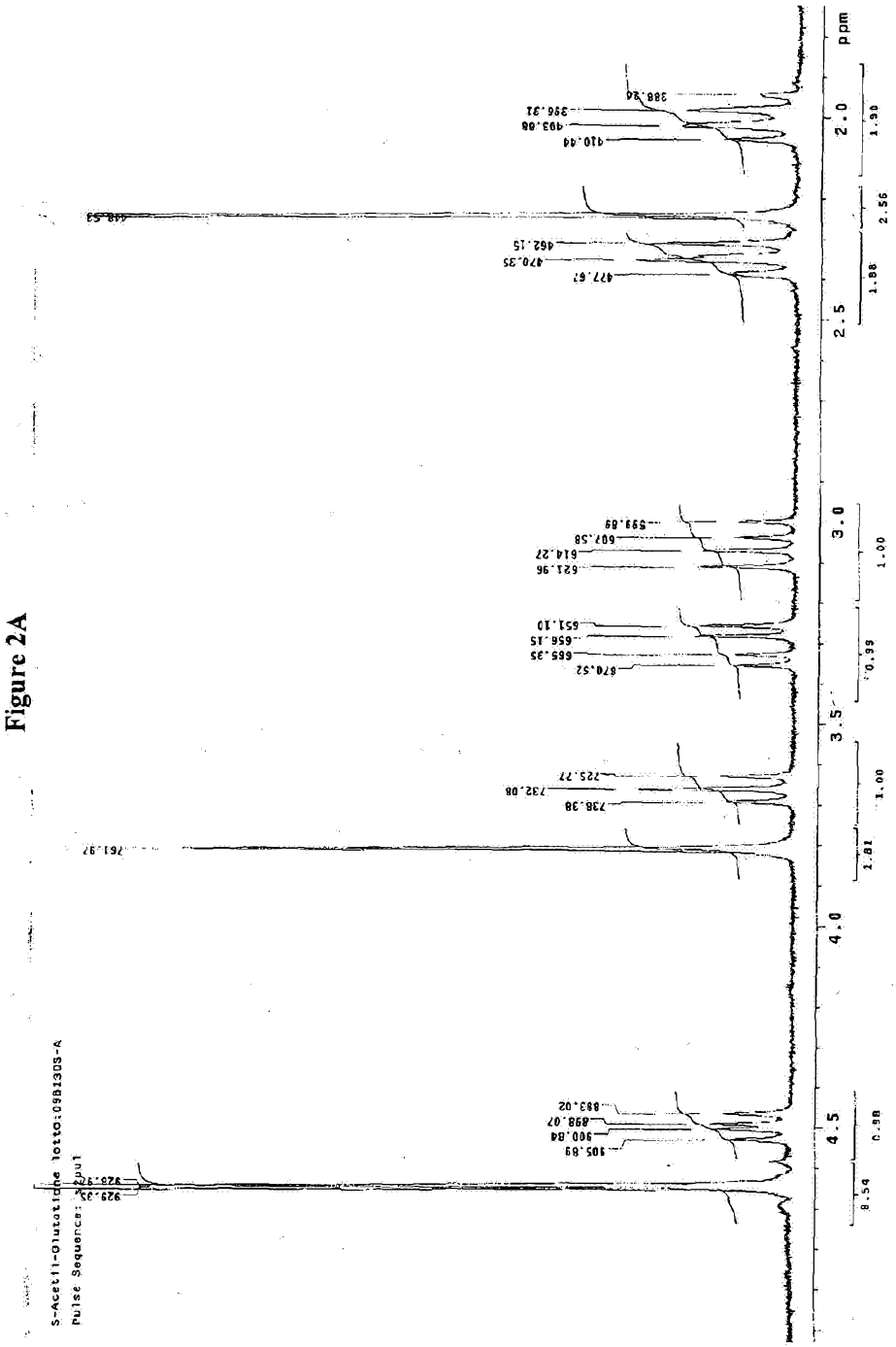

S-Acetil-Glutatione lotto:0981303-A
Pulse Sequence: zgpu1
ppm
4.5
4.0
3.5
3.0
2.5
2.0
1.90
2.56
1.88
1.00
0.99
1.00
1.81
0.98
8.54
448.53
462.15
470.35
477.67
599.89
607.58
614.27
621.96
651.13
656.15
665.35
670.52
725.27
732.08
738.38
761.97
883.02
898.07
900.84
905.89
929.33
388.36
396.31
493.08
410.44

Figure 3

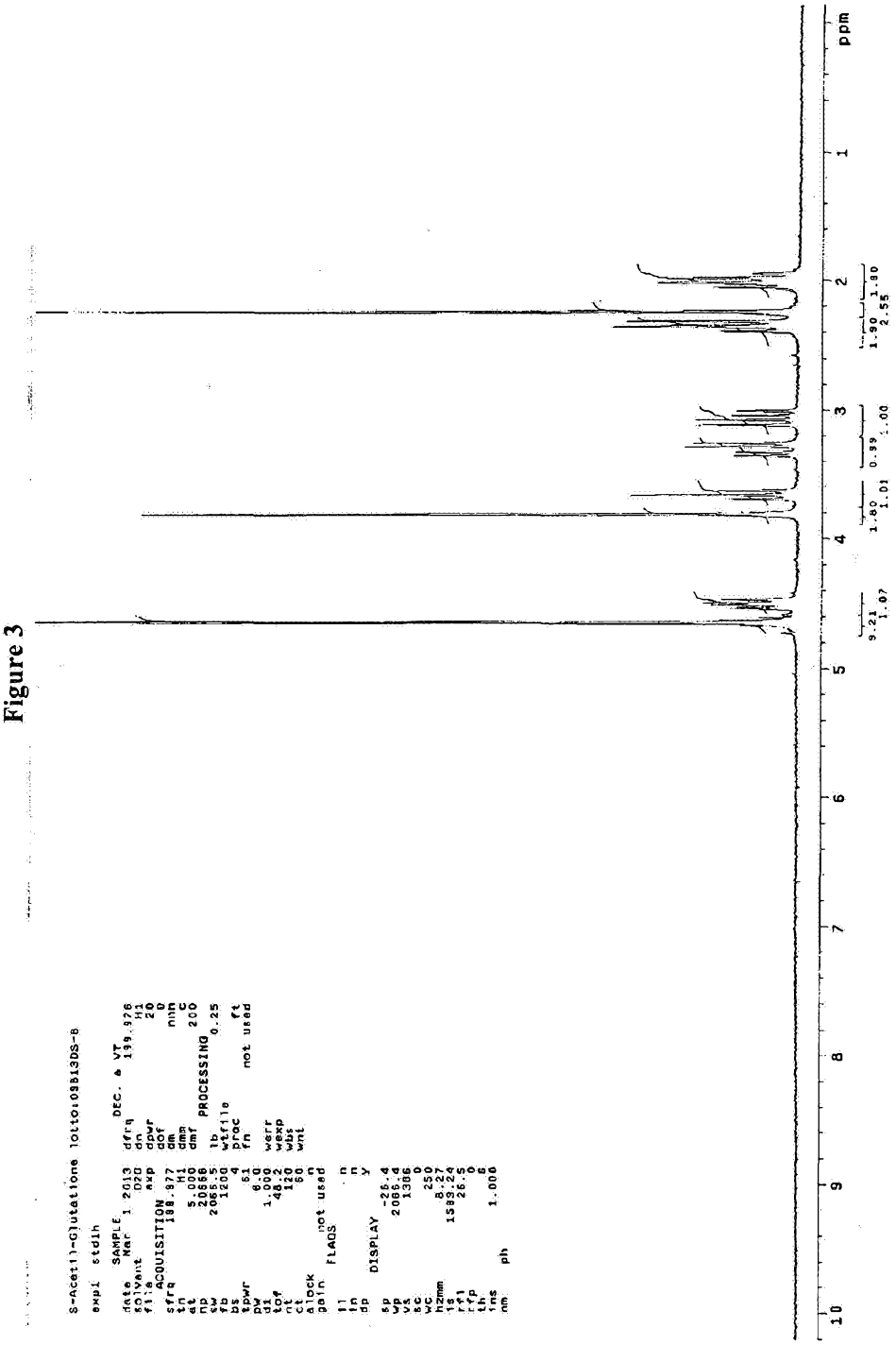


Figure 3A

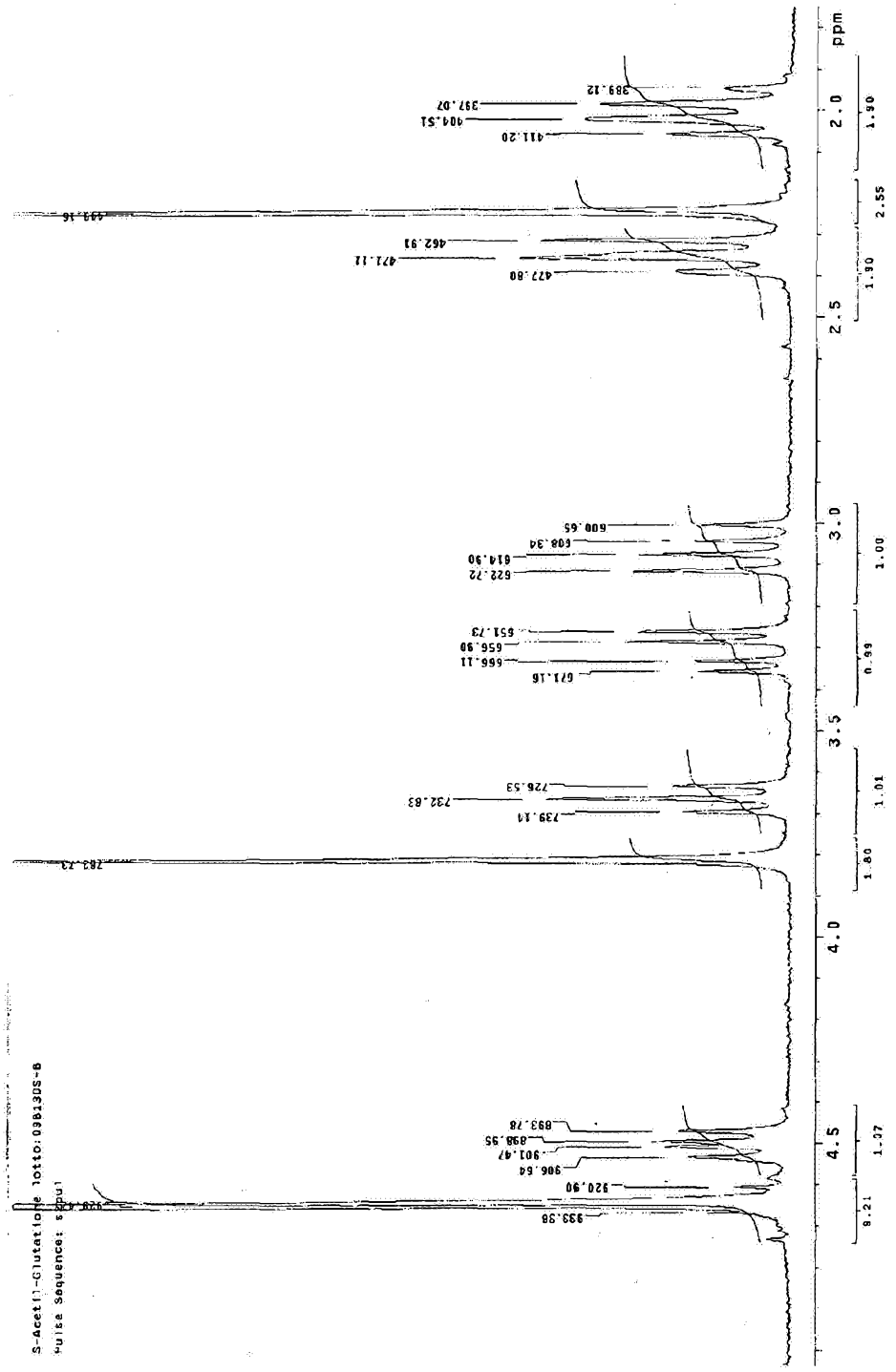

S-Acetil-Glutatione lotto:0381305-B
Pulse Sequence: s2pul
ppm
4.5
4.0
3.5
3.0
2.5
2.0
1.90
2.55
1.00
0.99
1.01
1.80
1.07
9.21
933.58
920.90
906.64
901.47
898.95
893.78
783.73
739.14
732.83
726.53
671.16
666.11
656.90
651.73
622.72
614.90
608.34
600.65
493.16
472.11
462.93
427.80
411.20
404.51
397.07
389.12

Figure 4

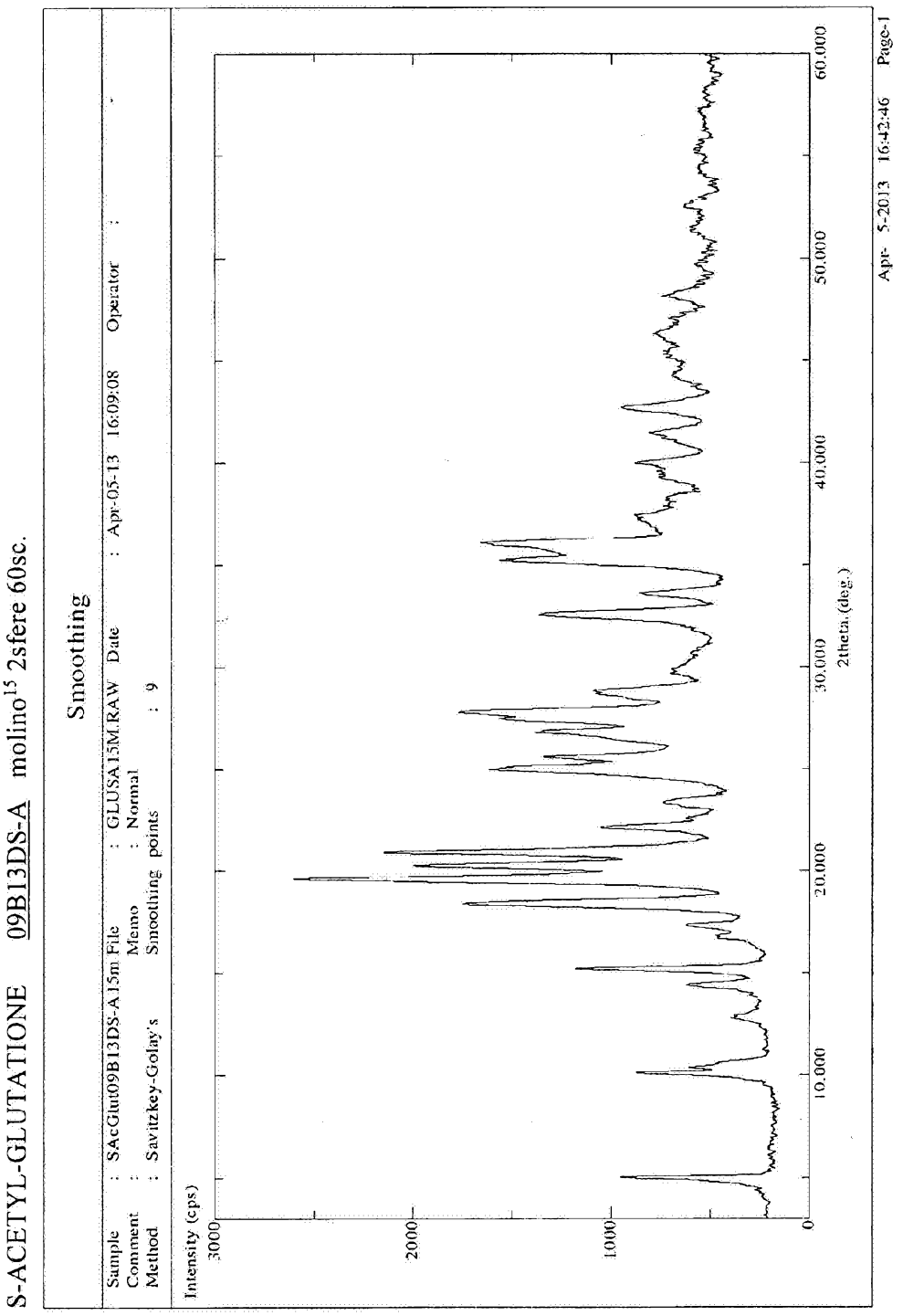

S-ACETYL-GLUTATIONE 09B13DS-A molino[15] 2sfere 60sc.
Smoothing
Sample : SAcGlut09B13DS-A15m File : GLUSA15M.RAW Date : Apr-05-13 16:09:08 Operator :
Comment : Memo : Normal
Method : Savitzkey-Golay's Smoothing points : 9
Intensity (cps)
3000
2000
1000
0
10.000
20.000
30.000
40.000
50.000
60.000
2theta (deg.)
Apr- 5-2013 16:42:46 Page-1

Figure 5

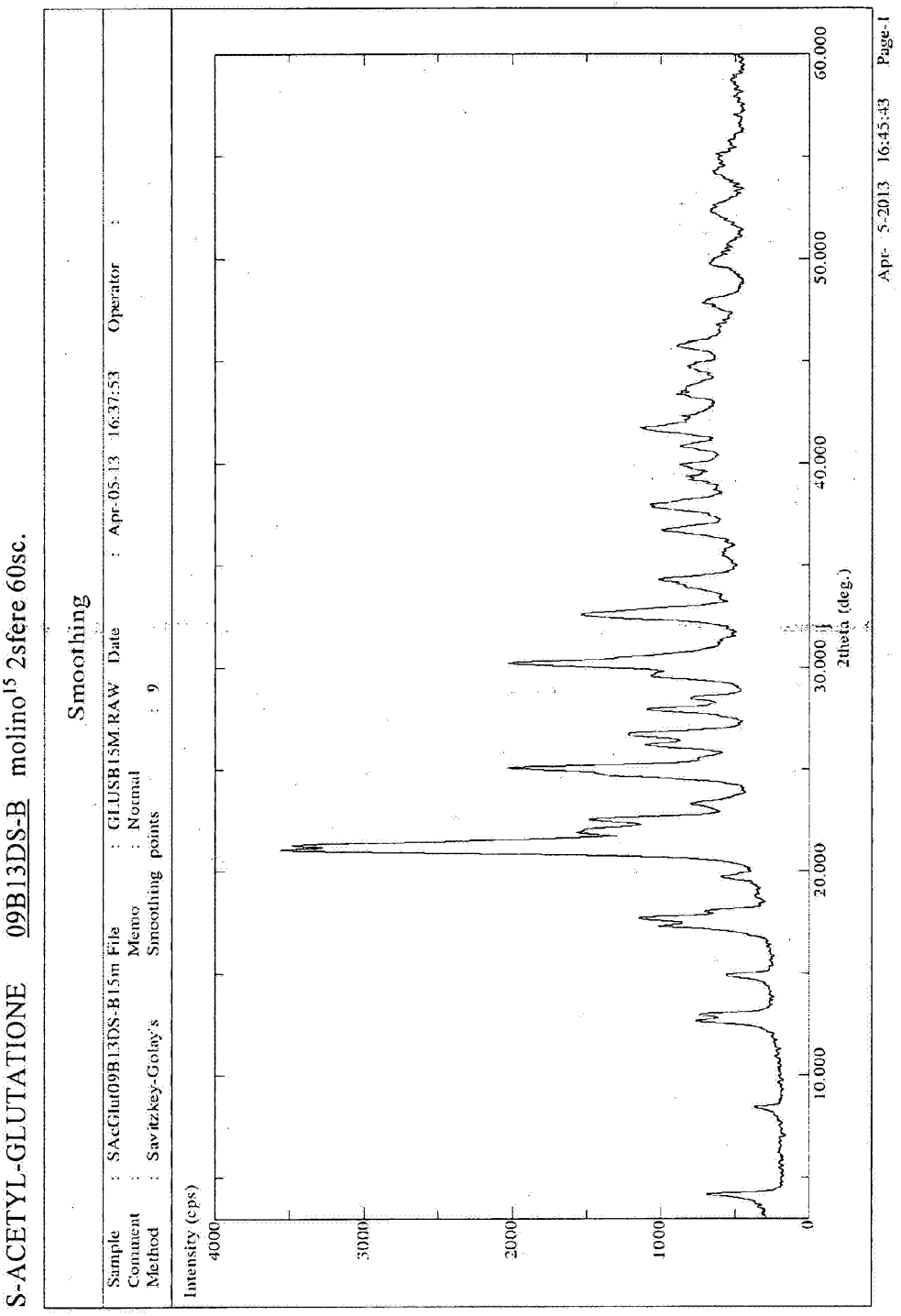
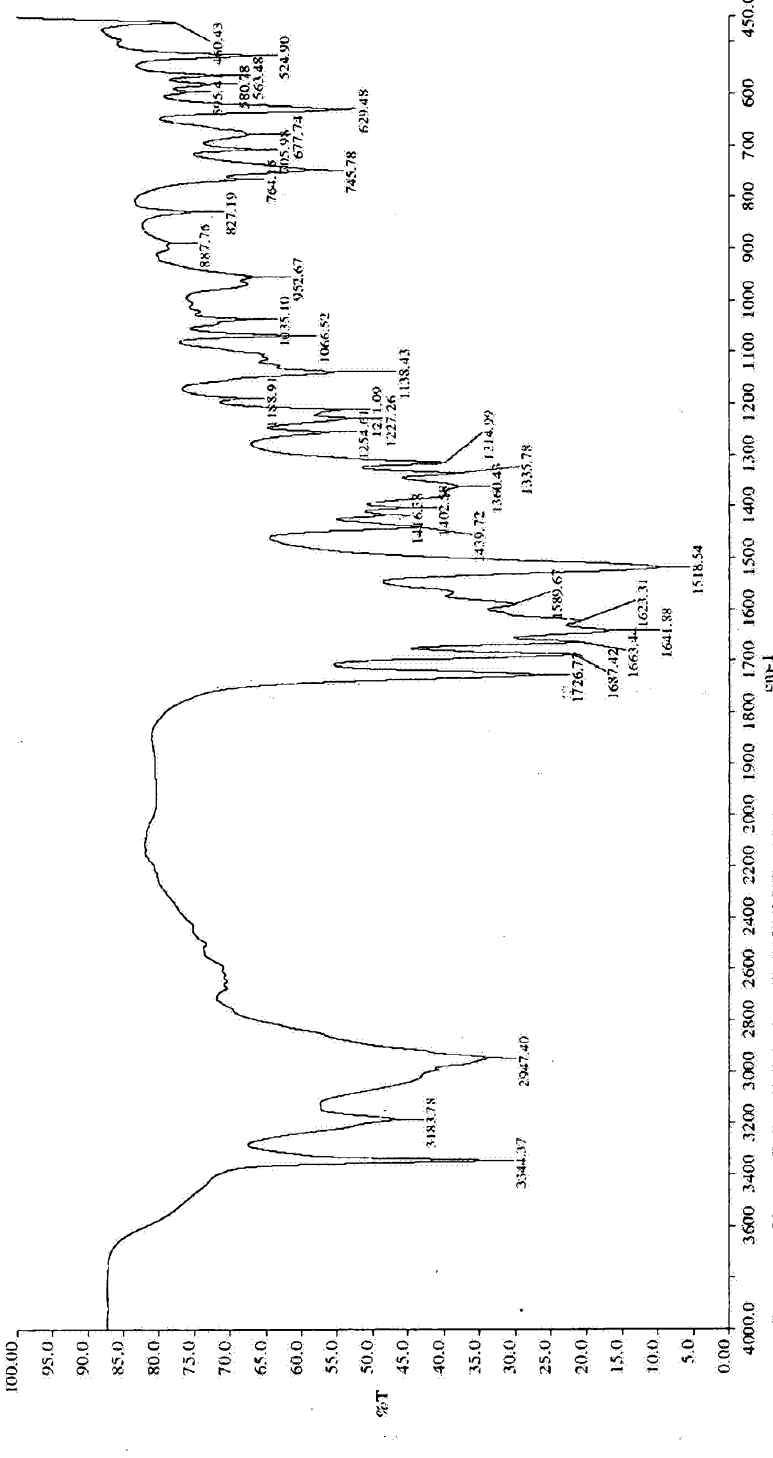

S-ACETYL-GLUTATIONE 09B13DS-B molino[15] 2sfere 60sc.
Smoothing
Sample : SAcGlut09B13DS-B15m File : GLUSB15M.RAW Date : Apr-05-13 16:37:53 Operator :
Comment : Memo : Normal
Method : Savitzkey-Golay's Smoothing points : 9
Intensity (cps)
4000
3000
2000
1000
0
10.000
20.000
30.000
40.000
50.000
60.000
2theta (deg.)
Apr- 5-2013 16:45:43 Page-1

Figure 6

A



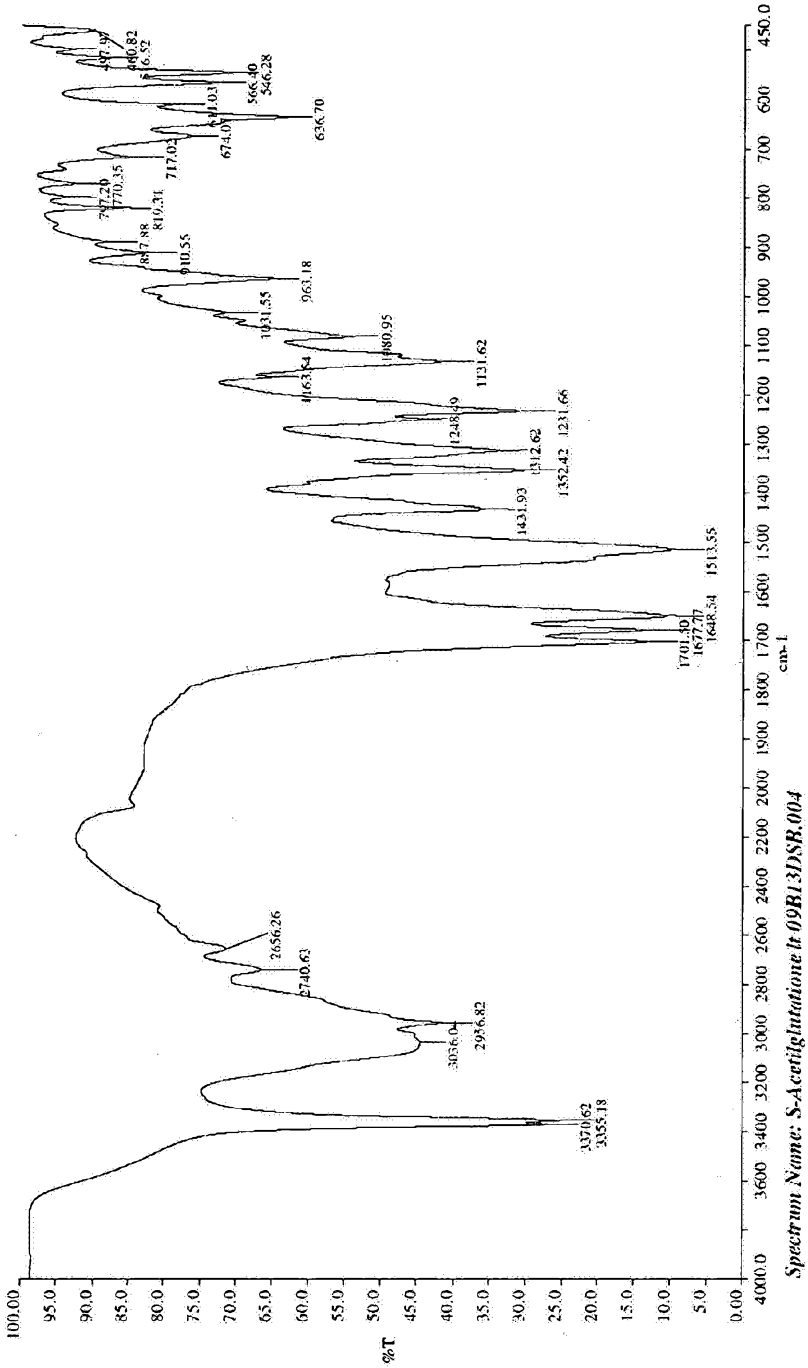
Spectrum Name: S-Acetilglutatione lt 09B13DSA.004

Description: S-Acetilglutatione lt 09B13DSA

Comments: Compressa KBr dil 1:100 senza vuoto

Figure 7

B



Spectrum Name: S-Acetilglutatione lt 09B13DSB.004

Description: S-Acetilglutatione lt 09B13DSB

Comments: Compressa KBr dil 1:100 senza vuoto

Figure 8

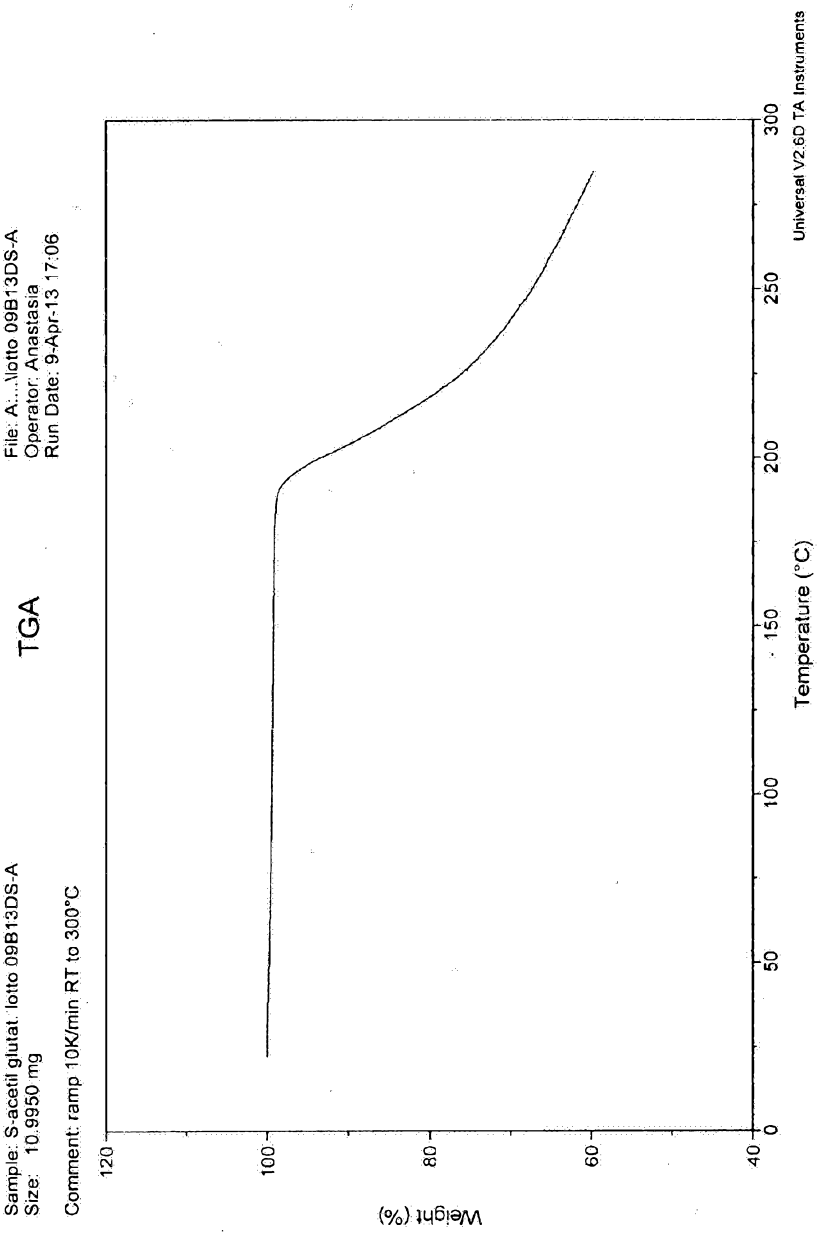

TGA
File: A:...\lotto 09B13DS-A
Operator: Anastasia
Run Date: 9-Apr-13 17:06
Sample: S-acetil glutat. lotto 09B13DS-A
Size: 10.9950 mg
Comment: ramp 10K/min RT to 300°C
Weight (%)
120
100
80
60
40
0
50
100
150
200
250
300
Temperature (°C)
Universal V2.6D TA Instruments

Figure 9

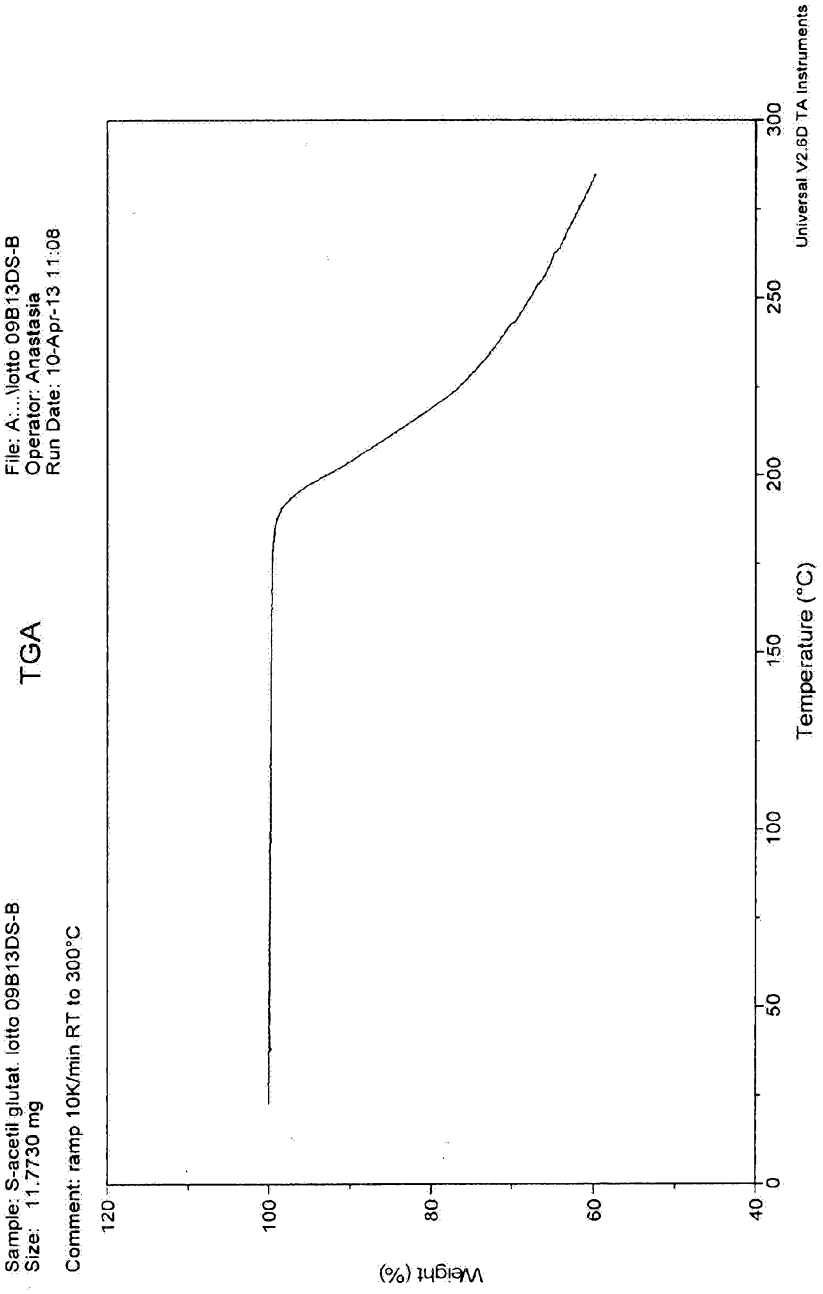

Sample: S-acetil glutat. lotto 09B13DS-B
Size: 11.7730 mg
Comment: ramp 10K/min RT to 300°C
TGA
File: A:...\lotto 09B13DS-B
Operator: Anastasia
Run Date: 10-Apr-13 11:08
Universal V2.6D TA Instruments
Weight (%)
Temperature (°C)
120
100
80
60
40
0
50
100
150
200
250
300

Figure 10

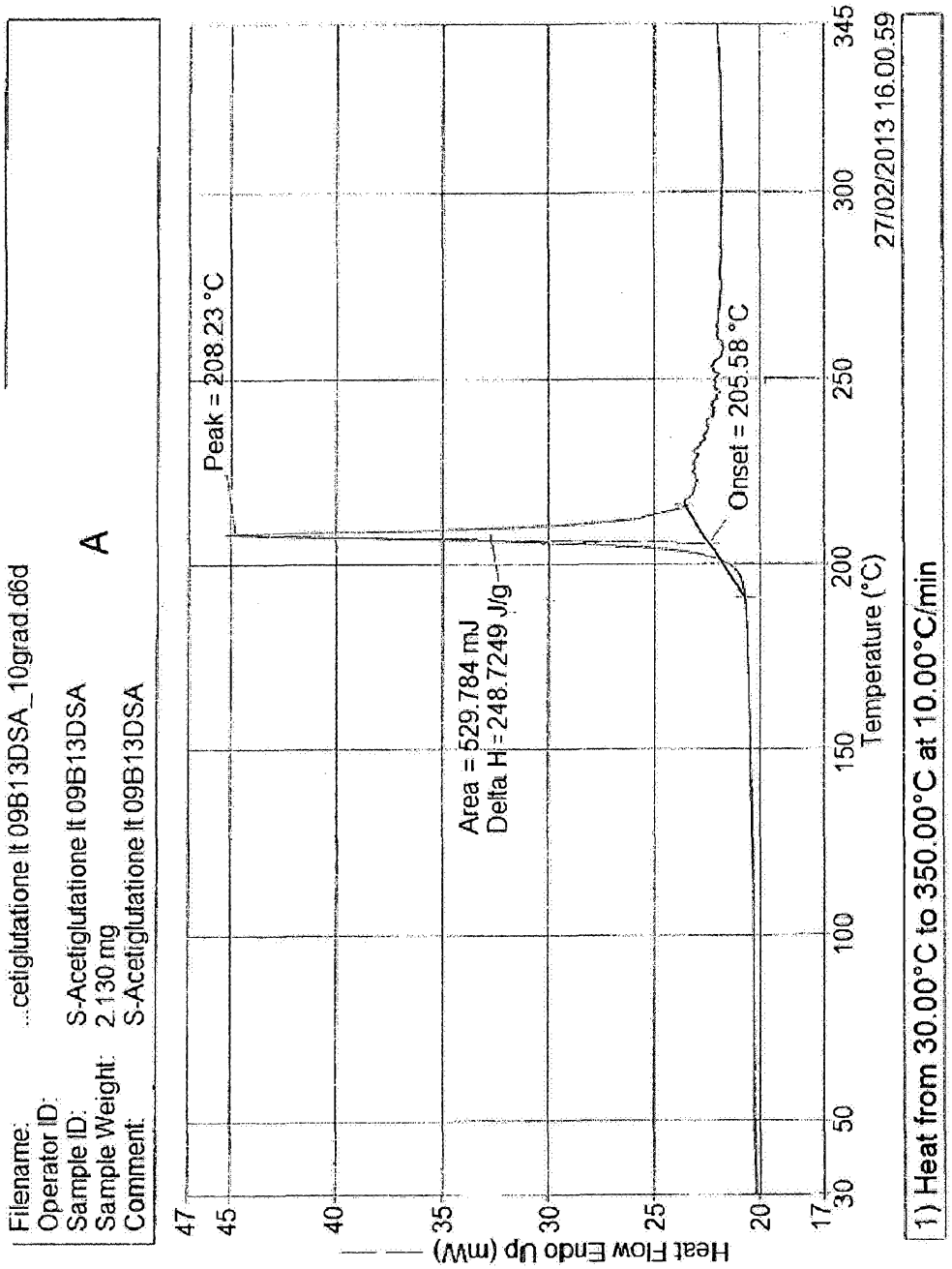

Filename: ...cetiglutatione lt 09B13DSA_10grad.d6d
Operator ID:
Sample ID: S-Acetiglutatione lt 09B13DSA
Sample Weight: 2.130 mg
Comment: S-Acetiglutatione lt 09B13DSA
A
Peak = 208.23 °C
Onset = 205.58 °C
Area = 529.784 mJ
Delta H = 248.7249 J/g
Heat Flow Endo Up (mW)
Temperature (°C)
27/02/2013 16.00.59
1) Heat from 30.00°C to 350.00°C at 10.00°C/min

Figure 11

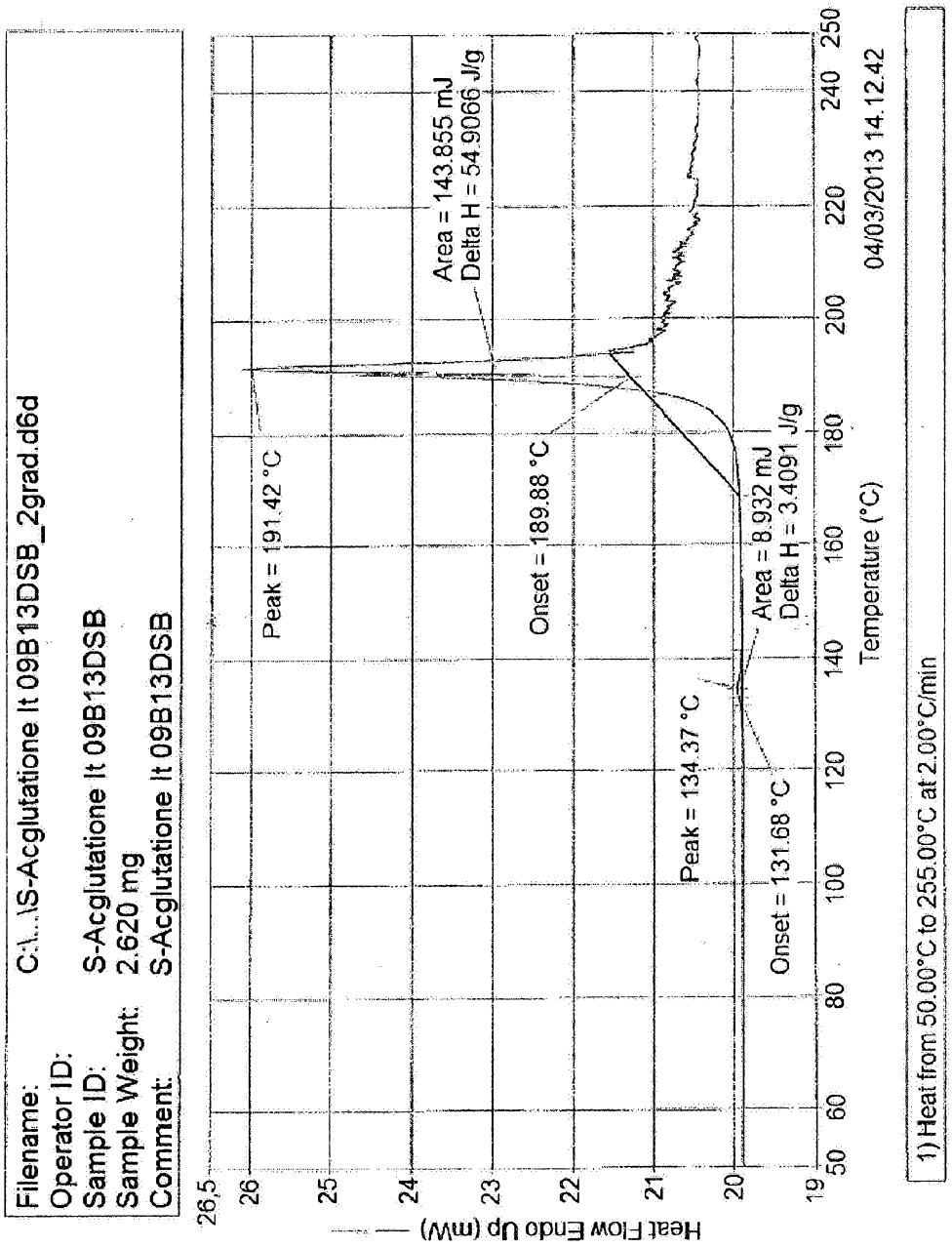
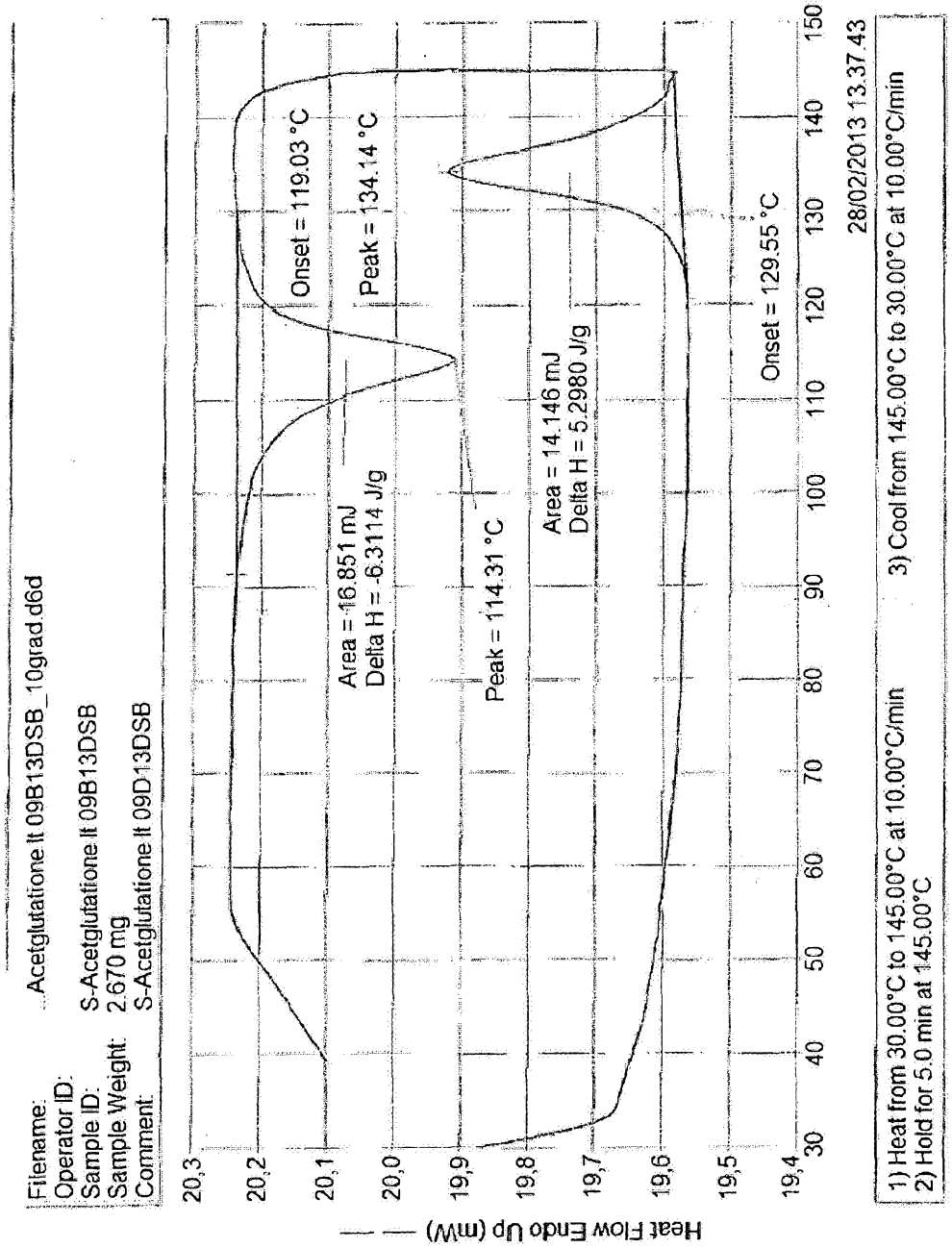

Filename: C:\...\S-Acglutatione It 09B13DSB_2grad.d6d
Operator ID:
Sample ID: S-Acglutatione It 09B13DSB
Sample Weight: 2.620 mg
Comment: S-Acglutatione It 09B13DSB
Heat Flow Endo Up (mW)
Temperature (°C)
04/03/2013 14.12.42
Peak = 191.42 °C
Onset = 189.88 °C
Area = 143.855 mJ
Delta H = 54.9066 J/g
Peak = 134.37 °C
Onset = 131.68 °C
Area = 8.932 mJ
Delta H = 3.4091 J/g
1) Heat from 50.00°C to 255.00°C at 2.00°C/min

Figure 12


Filename: ...Acetglutatione lt 09B13DSB_10grad.d6d
Operator ID:
Sample ID: S-Acetglutatione lt 09B13DSB
Sample Weight: 2.670 mg
Comment: S-Acetglutatione lt 09D13DSB
Onset = 119.03 °C
Peak = 134.14 °C
Area = 16.851 mJ
Delta H = -6.3114 J/g
Peak = 114.31 °C
Area = 14.146 mJ
Delta H = 5.2980 J/g
Onset = 129.55 °C
Heat Flow Endo Up (mW)
1) Heat from 30.00°C to 145.00°C at 10.00°C/min
2) Hold for 5.0 min at 145.00°C
3) Cool from 145.00°C to 30.00°C at 10.00°C/min
28/02/2013 13.37.43