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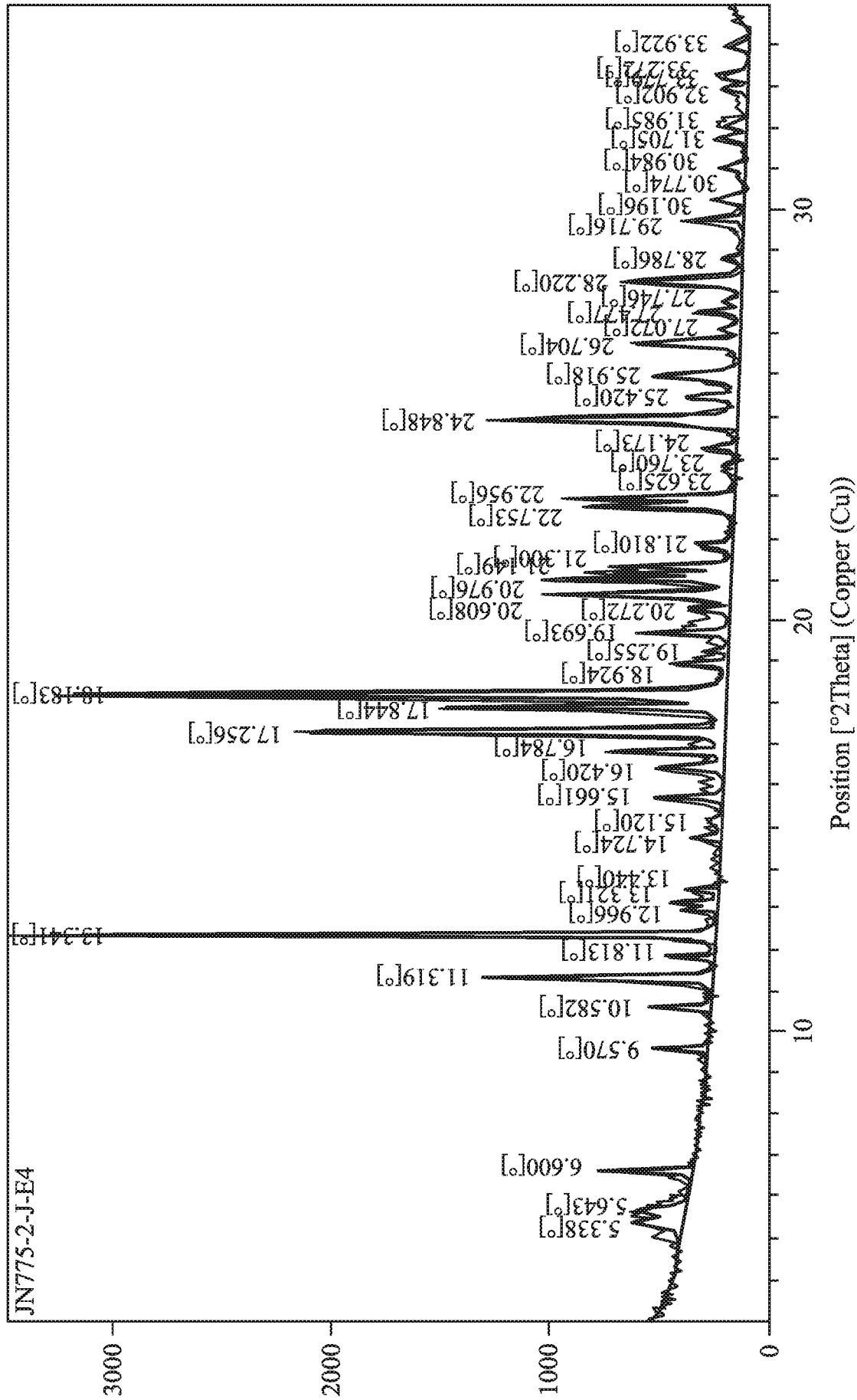
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Figure 1

18 09 18

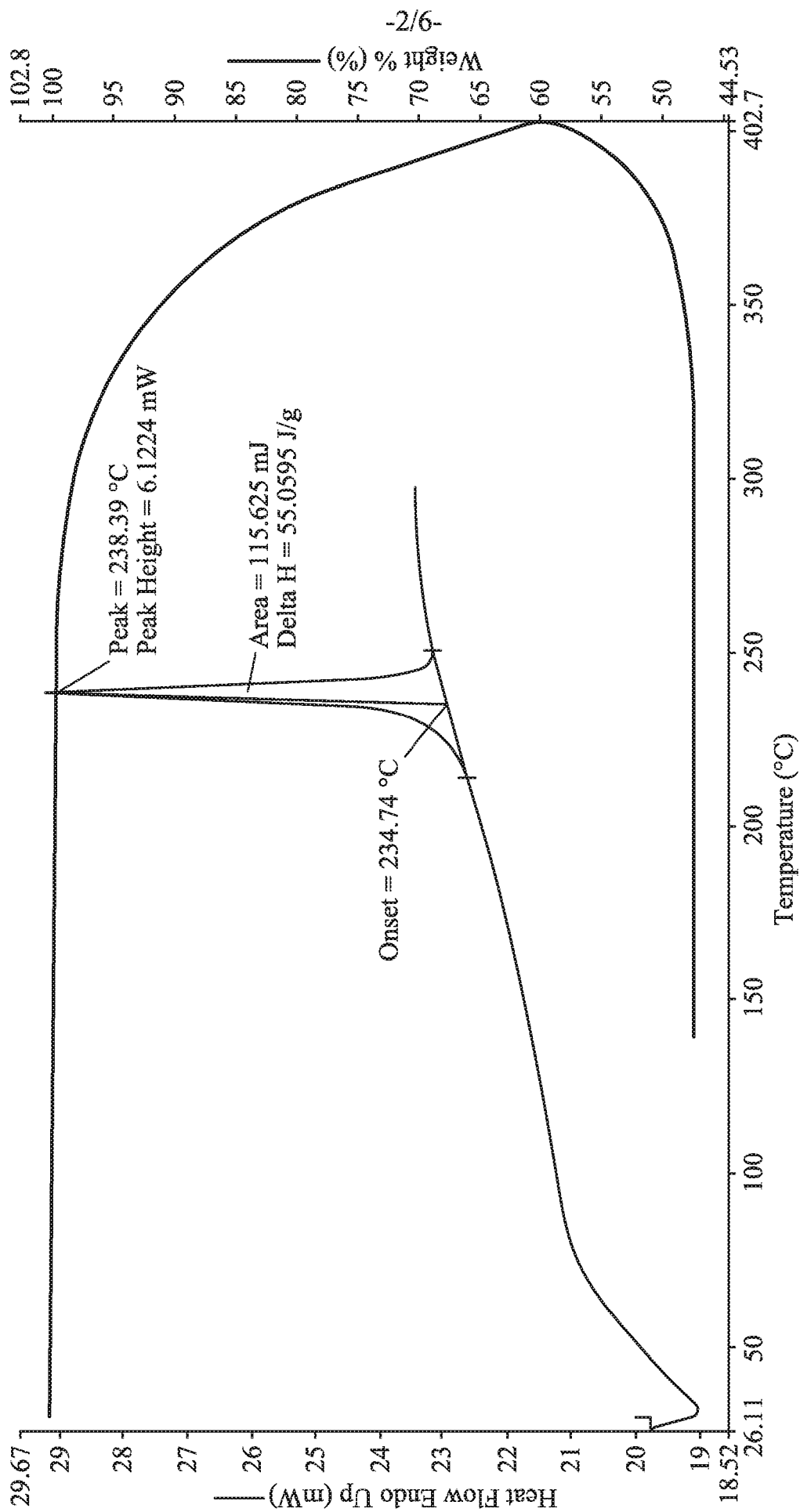
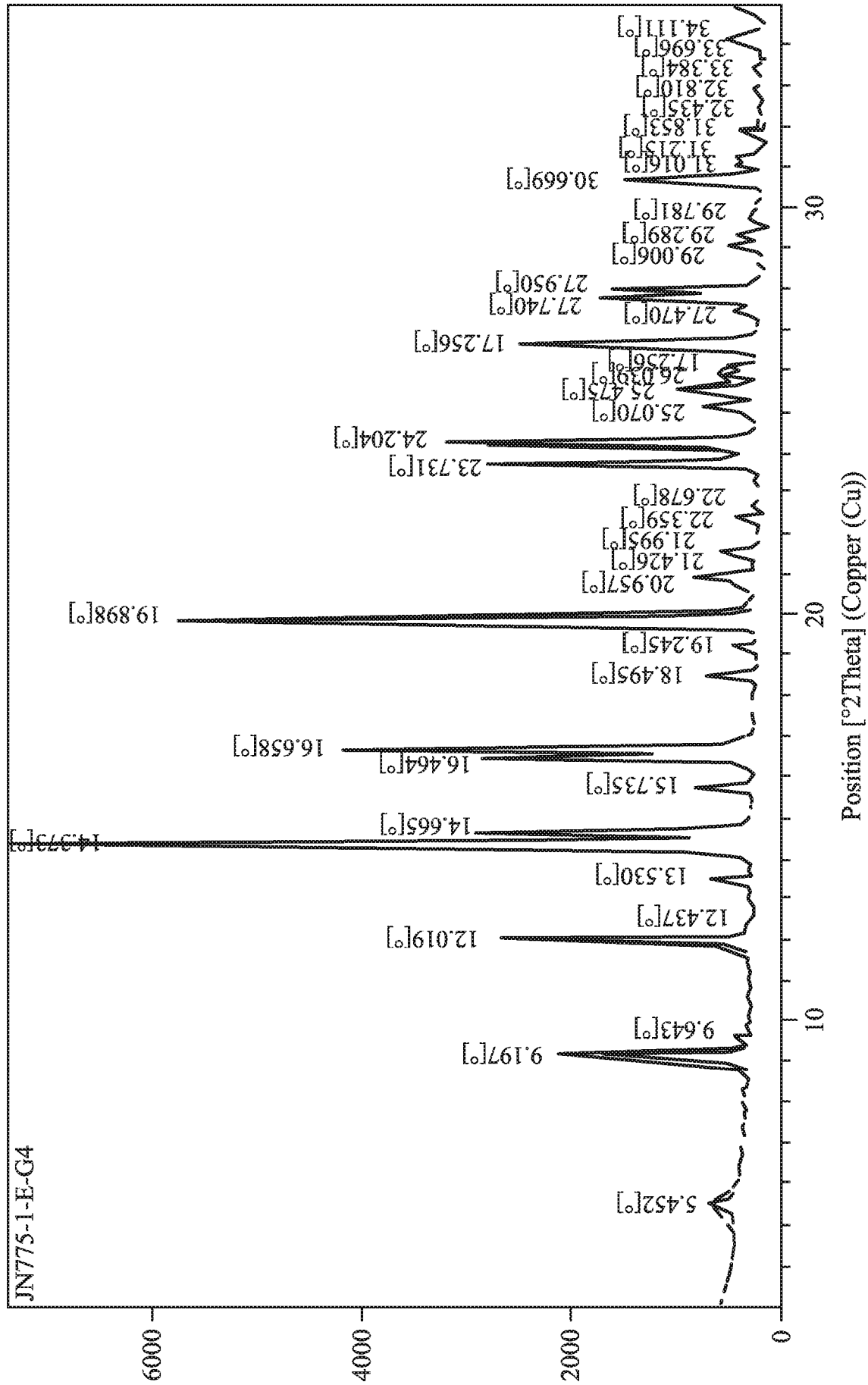


Figure 2

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Figure 3

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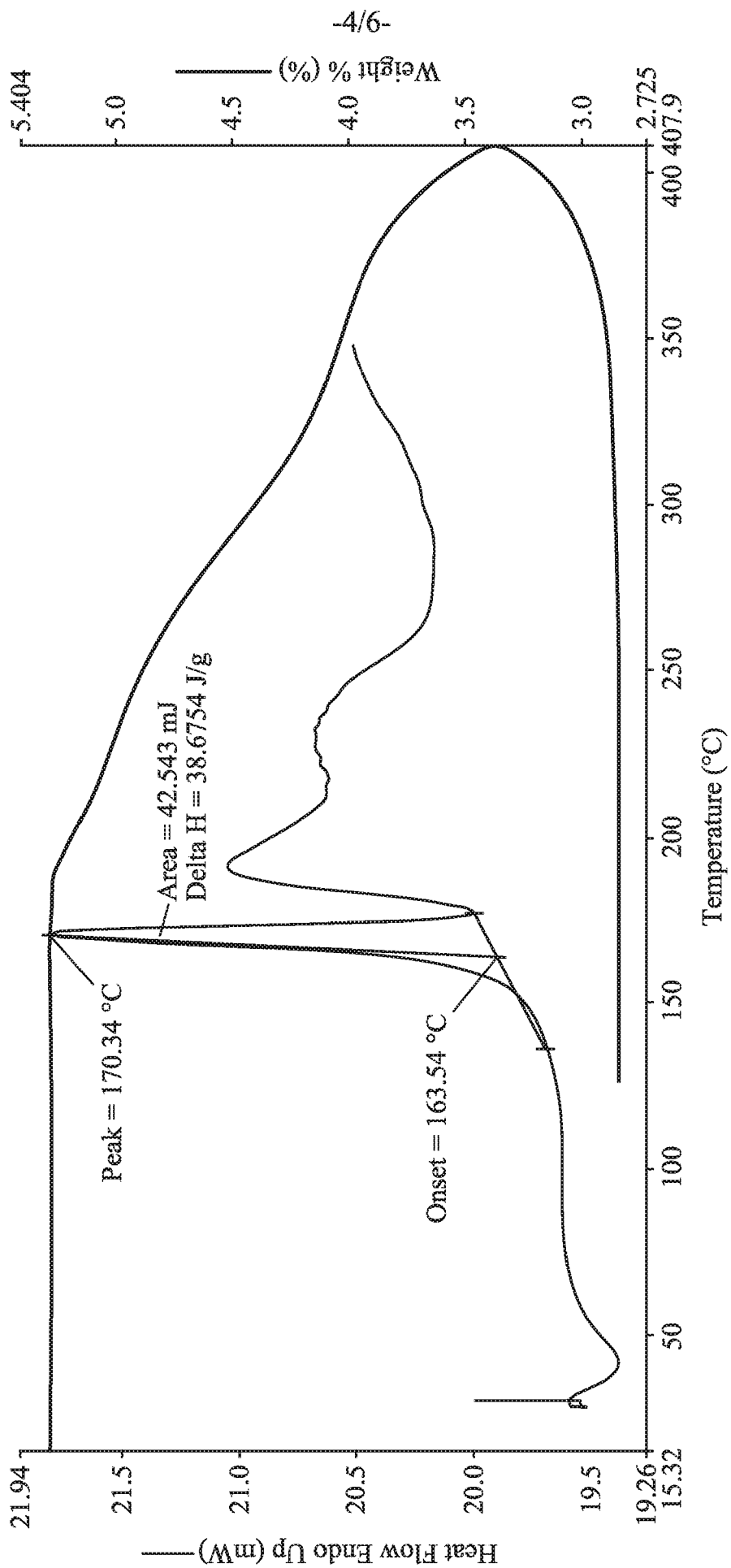


Figure 4

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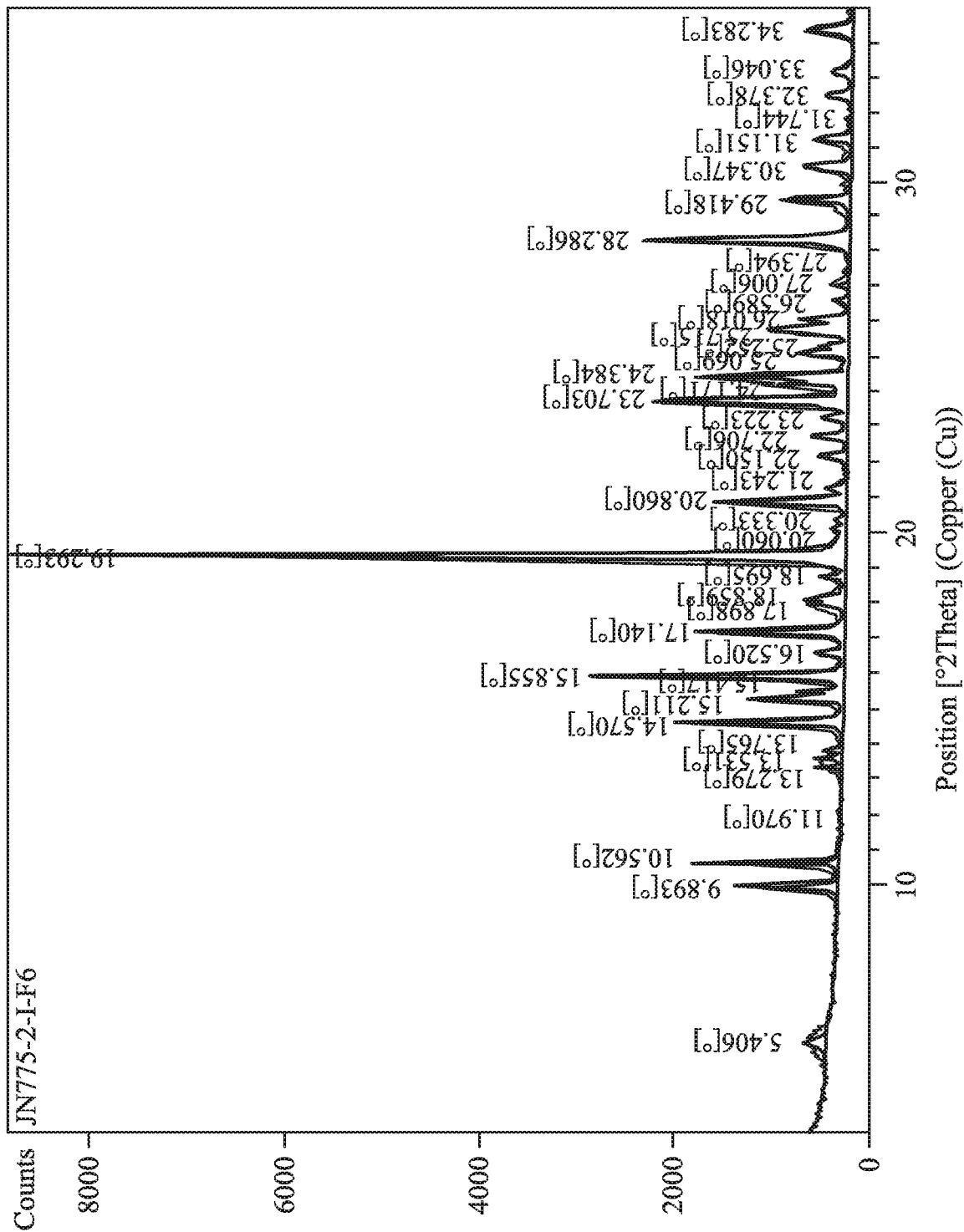


Figure 5

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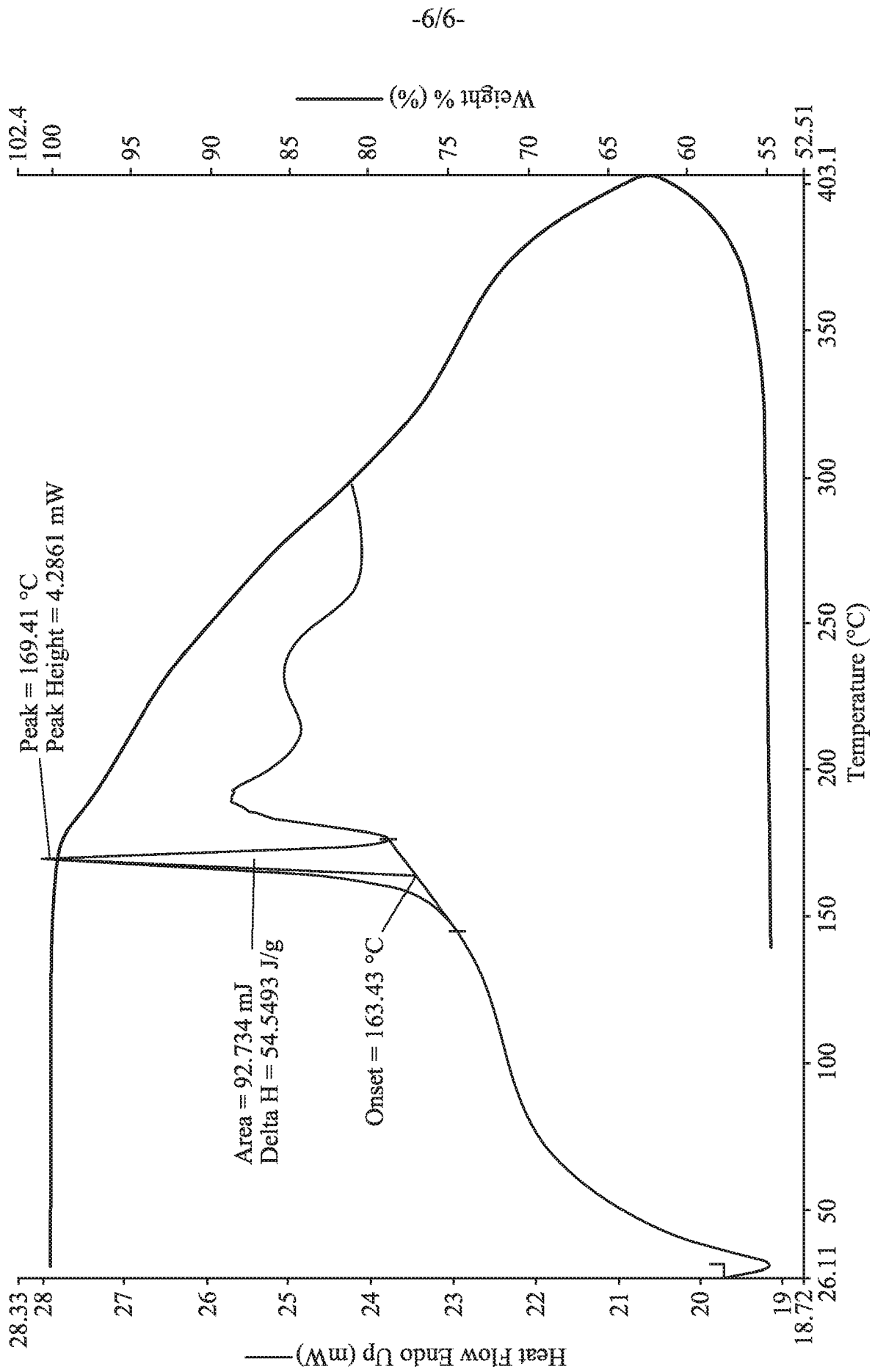


Figure 6

Salts

The present invention relates to novel salts of cytosine, processes for making them, pharmaceutical compositions containing them, and uses thereof.

Background to the Invention

Cytosine is a pyridone-like alkaloid known to be a potent nicotinic acetylcholine receptor agonist. Pharmacologically, cytosine exhibits a high degree of similarity to nicotine. Numerous studies have indicated that cytosine is useful in the treatment of nicotine addiction.

A pharmaceutical smoking cessation product containing cytosine has been commercialised for several years under the brand name TABEX®. The TABEX® product is marketed in the form of an orally administered tablet comprising 1.5mg of cytosine as a free base. While the product has been found to be efficacious and has been commercially successful, the approved shelf life of the product is two years.

As those skilled in the art will recognise, although a two-year shelf life for a pharmaceutical product is generally acceptable, it does impose pressure on the manufacturer and supply chain in terms of promptly packaging, transporting and delivering the product to users, and also increases the risk of stock wastage if the given shelf-life is exceeded. Additionally, where the product is to be shipped to territories with higher temperature or higher humidity climates (e.g. ICH climactic zones III and IV), then additional packaging or climate controls to protect the product and maintain shelf-life may be required.

A number of formulation approaches for cytosine-containing products are proposed in the prior art.

For example, EP1586320 discloses a solid dosage formulation of cytosine for oral administration. The formulation contains cytosine free base, lactose, microcrystal cellulose, talc powder, magnesium stearate and a film coating. Specific value ranges are given for the amount of cytosine and ratio of lactose:microcrystal cellulose, such compositions being said to provide tablets in which the active substance is evenly distributed. The results of stability tests are presented in the Examples. EP1586320 is concerned only with the free base of cytosine and does not consider cytosine salts.

WO2014/076680 relates to a solid dosage form in the form of a hard capsule comprising micronized cytosine and ancillary excipients. The formation of a homogeneous mass of

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powdered substances dosed into hard gelatine capsules containing a homogeneously dispersed active ingredient, prepared without the use of increased moisture and temperature is said to ensure cytosine stability throughout the technological process. WO2014/076680 discusses incompatibility between the cytosine active ingredient and lactose, and states that lactose may destabilise tablets comprising cytosine due to the presence of a carboxyl group in the lactose molecule, which is not completely inert chemically and may lead to a Maillard reaction. Salts of cytosine are not considered.

The succinate salt of cytosine is known from WO2017/134468. The salt may be formulated into a stable pharmaceutical composition comprising the succinate salt of cytosine and a pharmaceutically acceptable carrier which may be, for example, lactose monohydrate or anhydrous lactose. Improved stability of such compositions over compositions of cytosine free base and lactose is demonstrated.

The formation of salts of cytosine is known to be challenging, and no commercial formulations containing salts of cytosine are currently available. As demonstrated below, Cytosine does not form stable salts with common salt formers, such as acetic and benzoic acid, as demonstrated below. Surprisingly, however, the present inventors have found that stable salts of cytosine may be achieved when the salt is selected from a mesylate salt of cytosine; a gentisate salt of cytosine; and a maleate salt of cytosine. Such stable salts present additional options for formulating cytosine into stable pharmaceutical compositions.

Disclosure of the Invention

According to a first aspect of the present invention, there is provided a salt of cytosine selected from a mesylate salt of cytosine and a gentisate salt of cytosine. Such salts may be formulated with lactose or other common pharmaceutical carriers to provide compositions for use in therapy, for example, in the treatment of nicotine addiction.

The salts of the present invention are crystalline salts.

In embodiments of the present invention, the mesylate or gentisate salt of cytosine may be present in the form of a solvate or a hydrate.

In a second aspect of the present invention, there is provided a process for making a salt of cytosine selected from a mesylate salt of cytosine and a gentisate salt of cytosine, comprising:

- (i) preparing a first solution, of an acid salt former selected from methanesulphonic acid and gentisic acid;
- (ii) preparing a second solution, of cytosine;
- (iii) adding said solution of an acid salt former to said solution of cytosine;
- (iv) maintaining the solution obtained in step (iii) at an elevated temperature for a fixed period of time;
- (v) allowing the solution to cool to ambient temperature;
- (vi) filtering the cooled solution; and
- (vii) drying the obtained solids.

In the process of the invention, the first solution, of an acid salt former, may be provided for example by mixing the acid salt former in a suitable solvent, such as ethanol or a mixture of ethanol and water, for example. The second solution, of cytosine, may be prepared by dissolving cytosine in a suitable solvent, such as acetonitrile, or methyltetrahydrofuran, for example. Dissolution may be achieved by heating the solvent, for example to a temperature of 40-60°C, or by any other suitable method as will be known by the person skilled in the art. Once mixed in accordance with step (iii) above, the obtained solution may be maintained at an elevated temperature of, for example, around 40°C, for a time period of, for example, around one hour. After cooling, filtration of the obtained solids may be carried out, for example using a PTFE fritted column, and the obtained solids are then dried at a suitable temperature, for example at a temperature of around 50°C, for a suitable period of time, for example a period of time of around 48 hours.

The salts obtained in the above process may be further processed by known means, before being included in a pharmaceutical composition. For example, the salts may be micronized or otherwise processed.

According to a third aspect of the present invention, there is provided a pharmaceutical composition comprising a salt of cytosine selected from a mesylate salt of cytosine and a gentisate salt of cytosine, and a pharmaceutically acceptable carrier.

Pharmaceutical carriers are well known in the art. For example, lactose is commonly used in solid dosage formulations. In an embodiment, the pharmaceutically acceptable carrier in the compositions of the present invention is lactose. The lactose may be, for example, lactose monohydrate or anhydrous lactose. Alternative carriers include

cellulose, for example microcrystalline cellulose, or starch, for example corn starch and/or wheat starch.

Pharmaceutical compositions including cytosine salts in accordance with the present invention may have improved stability, for example in pharmaceutical compositions, and particularly in pharmaceutical compositions comprising lactose, compared with known compositions comprising cytosine free base.

The compositions disclosed herein may be suitable for administration by any route known in the art. Pharmaceutical compositions encompassed within this aspect of the invention include those suitable for oral, nasal or topical administration. In an embodiment, the composition may be formulated in a solid form such as a tablet or a capsule for oral administration.

The compositions of the present invention may additionally contain one or more excipients, such as are common in the art. Excipients that may be employed in the compositions of the present invention include, for example, fillers, disintegrants, preserving agents, lubricants and wetting agents.

Examples of fillers that may be used include lactose (for example, either anhydrous or monohydrate), cellulose, starch (for example, corn and / or wheat starch), calcium phosphates, calcium sulphates, and mannitol.

Preserving agents prevent bacterial or fungal contamination of the formulation and may include various antibacterial and antifungal agents such as parabens, chlorobutanol, phenol, and sorbic acid.

Suitable lubricants include stearic acid and its salts. One example of a lubricant for use in the compositions of the present invention is magnesium stearate.

The pharmaceutical compositions of the invention may further comprise sweetening, flavouring or colouring agents.

The skilled person will be well aware of suitable fillers, preserving agents and lubricants other than those specifically mentioned above, as well as suitable sweetening, flavouring, and colouring agents and other additives

The pharmaceutical compositions of the present invention may comprise a coating, for example a film coating, and may be coated according to any method known in the art, for example using collidone, shellac, gum arabic, talc, titanium dioxide, or sugar.

Pharmaceutical compositions in accordance with the present invention may be prepared by any suitable method. For example, capsules may be prepared by mixing the salt with one or more inert carriers such as lactose or sorbitol and packing into gelatine capsules. Tablets may be made by known compression methods, for example.

In embodiments of the invention, the pharmaceutical composition will be provided as a unit dosage form (e.g. a tablet or capsule). The amount of cytosine in the composition may range from about 0.5mg or about 1.0mg to 2.0mg, 3.0mg, 5.0mg or about 10mg. For example, a unit dosage form may comprise 1 to 3.5mg of the salt. In embodiments, the pharmaceutical compositions of the present invention may have a shelf life greater than 2 years when stored at 25°C and at a relative humidity of 60% ± 5%.

The salts and pharmaceutical compositions of the present invention are indicated for example for use in the treatment of nicotine addiction.

Thus, in a fourth aspect of the invention, there is provided a salt of cytosine selected from a mesylate salt of cytosine and a gentisate salt of cytosine, for use in the treatment of nicotine addiction, and a pharmaceutical composition comprising a salt of cytosine selected from a mesylate salt of cytosine and a gentisate salt of cytosine, and a pharmaceutically acceptable carrier, for use in the treatment of nicotine addiction.

In a fifth aspect of the present invention, there is provided a method of treating nicotine addiction in a subject, said method comprising administering to said subject an effective amount of a salt of cytosine selected from a mesylate salt of cytosine and a gentisate salt of cytosine.

Additionally disclosed herein (not part of the invention) is a maleate salt of cytosine.

Figures

Figure 1 shows the XRPD profile for cytosine mesylate.

Figure 2 shows the combined DSC/TGA profile for cytosine mesylate.

Figure 3 shows the XRPD profile for cytosine gentisate.

Figure 4 shows the combined DSC/TGA profile for cytosine gentisate.

Figure 5 shows the XRPD profile for cytosine maleate.

Figure 6 shows the combined DSC/TGA profile for cytosine maleate.

The various embodiments of the present invention will now be further explained with reference to the following examples.

Examples

Example 1 – Salt Formation

Stock solutions of acid salt formers were prepared in the carrier solvents and having the molarities detailed in the following Table 1 below:

Table 1 - List of acid solution for screening cytosine salt formation

Acid	Solvent mix	Molarity
Acetic	EtOH	1
Ascorbic	9:1 EtOH : Water	0.5
Benzoic	EtOH	1
Gentisic	EtOH	0.5
Glucuronic	Water	0.5
Maleic	EtOH	1
Methansulphonic	EtOH	1

Stock solutions of cytosine API (non-synthetic) were also prepared in CH₃CN and in Me-THF. 2.4g of cytosine was dissolved in 60ml of 2-Me-THF heated to a temperature of 60°C. 2.4g of cytosine was dissolved in 24ml of CH₃CN heated to a temperature of 40°C.

200ml capacity tubes heated to 40°C were charged with 2ml or 5ml of the cytosine stock solution. The acid stock solutions were then added to the heated tubes in equimolar amounts. The solutions were held at 40°C for one hour and then allowed to cool to ambient temperature (~18°C) for 18 hours. Where solid formation did not spontaneously occur, successive secondary manipulations were carried out in order to induce crystallisation, namely: i) gradual blow down under nitrogen to induce crystallisation, ii) charging antisolvent / triturate, and iii) second blow down under nitrogen and trituration with 3ml TBME and 1ml acetone.

Filtration of the obtained solids was then carried out using a PTFE fritted column and the obtained solids were dried at 50°C for 48 hours. The properties of the obtained products

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were then analysed, and the outcome of this screen and the properties of the obtained products are summarised in Table 2 below:

Table 2 – Salt preparation

API Stock Solution	Salt-type	Assessment
CH ₃ CN	Acetate	Secondary precipitations, unstable salt
2-MeTHF	Acetate	Secondary precipitations, unstable salt
CH ₃ CN	Ascorbate	Secondary precipitations, unstable salt
2-MeTHF	Ascorbate	Secondary precipitations, unstable salt
CH ₃ CN	Benzoate	Failed to yield solid
2-MeTHF	Benzoate	Failed to yield solid
CH ₃ CN	Gentisate	Excellent salt profile
2-MeTHF	Gentisate	Excellent salt profile
CH ₃ CN	Glucuronate	Failed to yield solid
2-MeTHF	Glucuronate	Failed to yield solid
CH ₃ CN	Maleate	Excellent salt profile
2-MeTHF	Maleate	Excellent salt profile
CH ₃ CN	Mesylate	Excellent salt profile
2-MeTHF	Mesylate	Excellent salt profile

As can be seen, suitable salts could not be formed with the common acid salt formers acetic acid and benzoic acid, as well as with the more exotic salt formers ascorbic acid and glucuronic acid. However, the mesylate, gentisate, and maleate salts were readily formed, exhibiting advantageous properties.

Example 2 – Characterisation Tests

The obtained salts were subjected to XRPD, DSC and TGA analysis, using the apparatus and parameters outlined below. The results are presented in Tables 3-5 below and illustrated in Figures 1-6 as described above.

X-Ray powder diffraction (XRPD)

X-Ray Powder Diffraction patterns were collected on a PANalytical^{RTM} diffractometer using Cu K α radiation (45kV, 40mA), θ - θ goniometer, focusing mirror, divergence slit (1/2"), soller slits at both incident and divergent beam (4mm) and a PIXcel^{RTM} detector.

The software used for data collection was X'Pert^{RTM} Data Collector, version 2.2f and the data was presented using X'Pert^{RTM} Data Viewer, version 1.2d.

XRPD patterns were acquired under ambient conditions via a transmission foil sample stage (polyimide - Kapton, 12.7 μ m thickness film) under ambient conditions using a PANalytical^{RTM} X'Pert^{RTM} PRO. The data collection range was 2.994 - 35 $^{\circ}$ 2 θ with a continuous scan speed of 0.202004 $^{\circ}$ s⁻¹.

Differential Scanning Calorimetry (DSC)

DSC data was collected on a PerkinElmer Pyris^{RTM} 6000 DSC equipped with a 45 position sample holder. The instrument was verified for energy and temperature calibration using certified indium. A predefined amount of the sample, 0.5-3.0mg, was placed in a pin holed aluminium pan and heated at 20 $^{\circ}$ C.min⁻¹ from 30 to 350 $^{\circ}$ C, or varied as experimentation dictated. A purge of dry nitrogen at 20ml.min⁻¹ was maintained over the sample. The instrument control, data acquisition and analysis were performed with Pyris^{RTM} Software v11.1.1 Revision H.

Thermo-Gravimetric Analysis (TGA)

TGA data were collected on a PerkinElmer Pyris^{RTM} 1 TGA equipped with a 20 position auto-sampler. The instrument was calibrated using a certified weight and certified Alumel and Perkalloy for temperature. A predefined amount of the sample, 1-5mg, was loaded onto a pre-tared aluminium crucible and was heated at 20 $^{\circ}$ C.min⁻¹ from ambient temperature to 400 $^{\circ}$ C. A nitrogen purge at 20ml.min⁻¹ was maintained over the sample. The instrument control, data acquisition and analysis were performed with Pyris^{RTM} Software v11.1.1 Revision H.

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Table 3 – Cytisine Mesylate Characterization

Properties	Cytisine Mesylate
XRPD	Crystalline. Peaks at: 5.3, 5.6, 6.6, 9.6, 10.6, 11.3, 11.8, 12.3, 13.0, 13.4, 14.7, 15.1, 15.7, 16.4, 16.8, 17.3, 17.8, 18.2, 18.9, 19.3, 19.7, 20.3, 20.6, 21.0, 21.3, 21.8, 22.8, 23.0, 23.6, 23.8, 24.2, 24.8, 25.4, 25.9, 26.7, 27.0, 27.5, 27.7, 28.2, 28.8, 29.7, 30.2, 30.8, 31.0, 31.7, 32.0, 32.9, 33.2, 33.3, 33.9 2 θ
DSC	Sharp endotherm at 238°C
TGA	Decomposition from 238°C

Table 4 – Cytisine Gentsiate Characterization

Properties	Cytisine Gentsiate
XRPD	Crystalline. Peaks at: 5.5, 9.2, 9.6, 12.0, 12.4, 13.5, 14.4, 14.7, 15.7, 16.5, 16.7, 18.5, 19.2, 19.9, 21.0, 21.4, 21.6, 22.4, 22.7, 23.7, 24.2, 25.1, 25.5, 25.9, 26.1, 26.6, 27.5, 27.7, 28.0, 29.0, 29.3, 29.8, 30.7, 31.0, 31.2, 31.9, 32.4, 32.8, 33.4, 33.7, and 34.1 2 θ
DSC	Sharp endotherm at 230°C
TGA	Decomposition from 230°C

Table 5 – Cytisine Maleate Characterization

Properties	Cytisine Maleate
XRPD	Crystalline. Peaks at: 5.4, 9.9, 10.6, 12.0, 13.3, 13.6, 13.8, 14.6, 15.2, 15.4, 15.9, 16.6, 17.1, 17.9, 18.1, 18.7, 19.3, 20.1, 20.3, 20.9, 21.2, 22.2, 22.7, 23.2, 23.7, 24.2, 24.4, 25.1, 25.3, 25.7, 26.0, 26.6, 27.0, 27.4, 28.3, 29.4, 30.3, 31.2, 31.8, 32.4, 33.0 and 34.3 2 θ
DSC	Sharp endotherm at 169°C
TGA	Decomposition from 169°C

Example 3 – Lactose Compatibility

The stability of formulations of cytosine and the present cytosine salts with lactose was investigated. Samples were stored at 25°C and 60% relative humidity (RH) and at 40°C and 75% RH and evaluated after 7 and 14 days. As can be seen from the data in Tables 6 and 7, when present in the form of its mesylate or gentisate salt, cytosine is degraded at a substantially lower rate than when present in free base form. Thus, these salts effectively improve the stability of cytosine and facilitates its formulation with compositions comprising lactose.

Table 6 – Stability evaluation of cytosine salts in the presence of lactose following storage at 25°C and 60% RH:

Formulation	Cytosine by HPLC, area % - 7 days	Cytosine by HPLC, area % - 14 days
Cytosine with lactose	98.77	94.99
Cytosine mesylate with lactose	99.54	99.66
Cytosine gentisate with lactose	97.98	98.37

Table 7 – Stability evaluation of cytosine salts in the presence of lactose following storage at 40°C and 75% RH:

Formulation	Cytosine by HPLC, area % - 7 days	Cytosine by HPLC, area % - 14 days
Cytosine with lactose	78.71	59.76
Cytosine mesylate with lactose	99.20	99.89
Cytosine gentisate with lactose	96.67	97.45

The invention is further defined with reference to the following claims.

Claims

1. A salt of cytosine, selected from a mesylate salt of cytosine and a gentisate salt of cytosine.
2. A solvate or hydrate of a salt according to Claim 1.
3. A process for making a salt of cytosine, selected from a mesylate salt of cytosine and a gentisate salt of cytosine, comprising:
 - (i) preparing a first solution, of an acid salt former selected from methanesulphonic acid and gentisic acid;
 - (ii) preparing a second solution, of cytosine;
 - (iii) adding said solution of an acid salt former to said solution of cytosine;
 - (iv) maintaining the solution obtained in step (iii) at an elevated temperature for a fixed period of time;
 - (v) allowing the solution to cool to ambient temperature;
 - (vi) filtering the cooled solution; and
 - (vii) drying the obtained solids.
4. A pharmaceutical composition comprising a salt according to Claim 1 or Claim 2 and a pharmaceutically acceptable carrier.
5. A pharmaceutical composition according to Claim 4, wherein the pharmaceutically acceptable carrier is selected from lactose, corn starch, wheat starch, microcrystalline cellulose, and mixtures thereof.
6. A pharmaceutical composition according to Claim 4 or Claim 5, wherein the composition is provided as a unit dosage form.
7. A pharmaceutical composition as claimed in Claim 6, wherein a unit dose comprises from 0.5 to 10 mg of the cytosine salt.

8. A pharmaceutical composition as claimed in Claim 6 or Claim 7, wherein a unit dose comprises from 1 to 3.5 mg of the cytosine salt.
9. A pharmaceutical composition according to any one of Claims 4 to 8, wherein the composition is in the form of a tablet or capsule.
10. The salt according to claim 1 or claim 2 or the pharmaceutical composition according to any one of Claims 4 to 9 for use in the treatment of nicotine addiction.
11. The salt according to claim 1 or claim 2 or the pharmaceutical composition according to any one of Claims 4 to 9, wherein the salt is a gentisate salt of cytosine.

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