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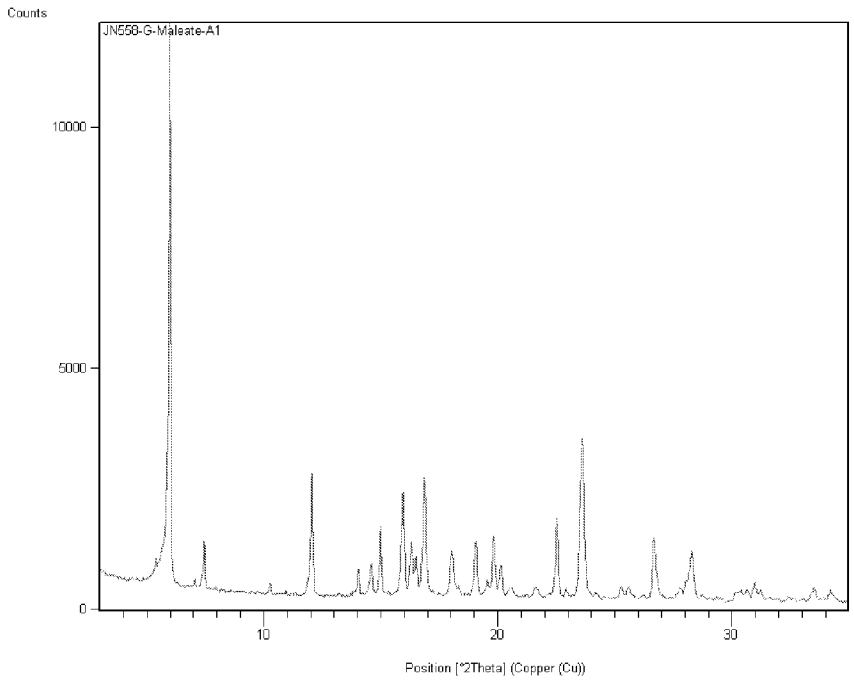

Figure 1

(57) Abstract: A liquid pharmaceutical composition for use in a pressurized metered dose inhaler (pMDI), which composition comprises a pharmaceutically acceptable addition salt of (i) carcainium, and (ii) sulfuric acid, hydrochloric acid, hydrobromic acid, maleic acid or 1-hydroxy-2-naphthoic acid(xinafoic acid).

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LIQUID PHARMACEUTICAL COMPOSITIONS

BACKGROUND OF THE INVENTION

Cough is the most common respiratory ailment for which patients seek medical help. It is a very common problem in medical practice as it accompanies a great variety of viral or bacterial infections including pneumonia, cold, flu and some underlying diseases, such as asthma, emphysema, lung cancer, etc.

Cough is a natural response to mechanical and chemical irritation of trachea and bronchi. The physiological role of cough is to prevent aspiration of foreign objects or excess secretion within the respiratory tract and to remove such objects or secretion or exudates from the trachea and bronchi.

Most of the current cough remedies are of limited effectiveness. Those that can be more effective are limited by their serious side effects. While there are several agents available on the market, most of these agents cause secondary undesirable symptoms, such as drowsiness, tiredness, gastrointestinal disturbances, and some of these agents, such as for example codeine, are also addictive.

Acute severe episodes of cough, although often limited in duration, can still be very troublesome. However, the condition of chronic cough (which persists in a troublesome form for more than eight weeks) is a serious debilitating condition estimated to affect some 14% of the population. It has an adverse effect on quality of life for many sufferers.

Over ten years ago, there was an initial suggestion that carcainium chloride might be useful in the treatment and/or prevention of cough. Thus, US 6,362,197, filed in 1999, sugggested that carcainium chloride might have this activity. That conclusion was based on experiments carried out in an animal model in which cough was induced by citric acid aerosol. US 6,362,197 did not contain any clinical data showing efficacy in treating cough in humans. Rather, the document simply assumed that clinical efficacy in humans would be achieved, based on the results from the animal model.

When carcainium chloride was first postulated as an anti-tussive, its structural similarity to known local anaesthetics was noted. It was assumed at the time that any anti-tussive activity was mediated by activity as a local anaesthetic. The expected side effects were thus local

oropharyngeal numbing, impairment or loss of gag reflex and/or impairment or loss of the tracheal aspiration reflex.

Shortly after US 6,362,197 was filed, it became apparent that the assumptions made in that document were not correct. Thus, a press release from Nortran Pharmaceuticals Inc. on 15 December 2000 reported the results of a Phase II clinical trial on carcainium chloride in healthy human volunteers. This clinical trial was a blinded placebo-controlled cross-over trial, in which the primary endpoint was to determine whether carcainium chloride could increase the amount of irritant required to induce cough in the subjects. This clinical trial clearly established that, contrary to the assumptions made in US 6,362,197, carcainium chloride had no statistically significant ability to inhibit cough. These clinical trial results rapidly became known in the art. Since 2000, it has been common general knowledge in this field that carcainium chloride does not have clinical efficacy in treating cough in humans.

SUMMARY OF THE INVENTION

It is a finding of the present invention that, contrary to the widespread understanding since 2000, carcainium salts can in fact provide an effective therapy for cough in humans. That conclusion is based on a new clinical trial in which carcainium chloride is seen to have a statistically significant therapeutic effect in treatment and/or suppression of cough in patients suffering from interstitial lung disease.

Pressurized metered dose inhalers are convenient devices for administering drugs to patients by inhalation, and rely on a propellant compound to generate an aerosol for inhalation. The propellant is commonly a hydrofluoroalkane. However, a pressurized metered dose inhaler can only be used to deliver a drug if that drug can formulated as a suspension or solution in the propellant. This can cause difficulties, since many drugs cannot be formulated as a suspension or solutions in propellants such as a hydrofluoroalkanes.

It is a further finding of the invention that certain specific salts of carcainium can be formulated as suspensions or solutions in propellants such as hydrofluoroalkanes. Such solutions or suspensions are suitable for delivery from pressurized metered dose inhalers.

Accordingly, the present invention provides a liquid pharmaceutical composition for use in a pressurized metered dose inhaler (pMDI), which composition comprises a pharmaceutically

acceptable addition salt of (i) carcainium, and (ii) sulfuric acid, hydrochloric acid, hydrobromic acid, maleic acid or 1-hydroxy-2-naphthoic acid (xinafoic acid).

The invention further provides a pressurized metered dose inhaler comprising a pharmaceutical composition of the invention.

The invention further provides a pharmaceutically acceptable addition salt of (i) carcainium, and (ii) sulfuric acid, hydrobromic acid, maleic acid or 1-hydroxy-2-naphthoic acid (xinafoic acid).

The invention further provides a pharmaceutical composition or carcainium salt of the invention, for use in the treatment of the human or animal body.

The invention further provides a pharmaceutical composition or carcainium salt of the invention, for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes.

The invention further provides use of pharmaceutical composition or carcainium salt of the invention in the manufacture of a medicament for the treatment and/or suppression of cough, tussive attacks or tussive episodes.

The invention further provides a method of treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, which method comprises administering to said patient a therapeutically effective amount of pharmaceutical composition or carcainium salt of the invention.

DESCRIPTION OF THE FIGURES

Figures 1 to 3 show the X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) patterns for carcainium maleate prepared in Example 1.

Figures 4 to 6 show the X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) patterns for carcainium oxalate prepared in Example 1.

Figures 7 to 9 show the X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) patterns for carcainium fumarate prepared in Example 1.

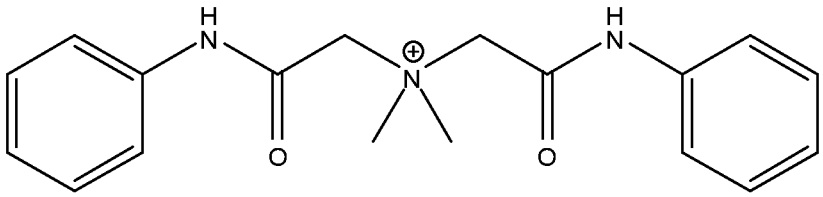
Figures 10 to 12 show the X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) patterns for carcainium sulfate prepared in Example 1.

Figures 13 to 15 show the X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) patterns for carcainium bromide prepared in Example 1.

Figure 16 shows the inhibition cough by carcainium salts in guinea pigs, as described in Example 5. Each bar represents the mean and the vertical lines represent standard error (SEM) for the guinea pigs tested.

DETAILED DESCRIPTION OF THE INVENTION

Carcainium is the compound N,N-Bis-(phenylcarbamoylmethyl) dimethylammonium and has the following structure.



The liquid pharmaceutical compositions of the invention comprise a pharmaceutically acceptable addition salt of (i) carcainium, and (ii) sulfuric acid, hydrochloric acid, hydrobromic acid or 1-hydroxy-2-naphthoic acid (xinafoic acid). The salt present in the composition is thus carcainium sulfate, carcainium chloride, carcainium bromide or carcainium 1-hydroxy-2-naphthoate (ie. carcainium xinafoate).

The carcainium sulfate salt typically has a stoichiometry of 2:1 carcainium:sulfate. The carcainium chloride salt typically has a stoichiometry of 1:1 carcainium:chloride. The carcainium bromide salt typically has a stoichiometry of 1:1 carcainium:bromide. The carcainium xinafoate salt typically has a stoichiometry of 1:1 carcainium:xinafoate. The stoichiometry of the salts can be determined by techniques known to those skilled in the art, such as ^{1}H NMR.

The liquid pharmaceutical compositions of the invention are for use in pressurized metered dose inhalers (pMDIs). The liquid pharmaceutical compositions therefore comprise a

propellant. The propellant provides the force to generate the aerosol cloud when the pMDI is actuated.

Suitable propellants can be selected by those skilled in the art. Typically the propellant is a hydrofluoroalkane. Preferred propellants are 1,1,1,2,-tetrafluoroethane (HFA 134a) and 1,1,1,2,3,3,3-heptafluoropropane (HFA 227) or mixtures thereof. Thus, the liquid pharmaceutical compositions of the invention typically comprises 1,1,1,2,-tetrafluoroethane and/or 1,1,1,2,3,3,3-heptafluoropropane. Preferably, the liquid pharmaceutical composition contains 1,1,1,2,-tetrafluoroethane as the sole propellant, or 1,1,1,2,3,3,3-heptafluoropropane as the sole propellant, or a 1:1 mixture of 1,1,1,2,-tetrafluoroethane and 1,1,1,2,3,3,3-heptafluoropropane as the sole propellant.

The carcainium salt is typically suspended or dissolved in the propellant. The liquid composition is thus typically a suspension of the carcainium salt or a solution of the carcainium salt.

If the liquid pharmaceutical composition is a suspension of the carcainium salt, then the carcainium salt is typically carcainium sulfate, carcainium chloride or carcainium bromide, preferably carcainium sulfate or carcainium chloride, most preferably carcainium sulfate.

The fine particle fraction of the particles in the suspension of carcainium salt is preferably greater than 10%, more preferably greater than 15%. The FPF is the proportion of particles which are less than 5μm in size. The FPF can readily be determined by techniques known to those skilled in the art, for example using an impactor.

If the liquid pharmaceutical composition is a solution of the carcainium salt, then the carcainium salt is typically carcainium xinafoate, carcainium maleate, carcainium chloride or carcainium bromide, preferably carcainium xinafoate, carcainium maleate, or carcainium chloride, more preferably carcainium xinafoate or carcainium maleate, most preferably carcainium xinafoate.

The liquid pharmaceutical composition of the invention optionally comprises additional components. For example, the liquid pharmaceutical composition may optionally comprise a surfactant (such as TWEEN) or an organic solvent (such as ethanol).

The liquid pharmaceutical composition of the invention is preferably delivered to a patient using a pressurized metered dose inhaler. The invention thus also relates to a pressurized metered dose inhaler comprising the liquid pharmaceutical composition of the invention.

The aerosol delivered from the pressurized metered dose inhaler typically has droplets which have a mass median aerodynamic diameter (MMAD) of from about 1 μm to about 10 μm, preferably from about 3 μm to about 10 μm, more preferably from about 4 μm to about 5.5 μm, or from about 5 μm to about 10 μm, or from about 5.5 μm to about 10 μm or from about 6 μm to about 10 μm. The mass median aerodynamic diameter (MMAD) can be measured by any suitable technique known to those skilled in the art, such as laser diffraction. Such particle sizes are preferred for effective delivery of the drug into the conducting and central airways.

The pharmaceutical compositions and carcainium salts of the invention are useful for the treatment and/or suppression of cough, tussive attacks or tussive episodes, preferably in a human patient.

Carcainium salts have efficacy as anti-tussives in human patients at dosages at which the salts have no local anaesthetic activity. Accordingly, the present invention also provides a said pharmaceutical composition or carcainium salt for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, wherein said carcainium salt acts by a mechanism independent of local anaesthesia.

The invention also provides a pharmaceutical composition or carcainium salt of the invention for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, without causing any substantial local anaesthetic effect. Local anaesthetic activity in inhaled medicaments causes side effects such as oropharyngeal numbing, impairment or loss of gag reflex and/or impairment or loss of the tracheal aspiration reflex. Typically, therefore, said carcainium salt is for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, without causing any substantial oropharyngeal numbing, impairment or loss of gag reflex and/or impairment or loss of the tracheal aspiration reflex.

Typically, said pharmaceutical composition or carcainium salt is for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient suffering from or susceptible to bronchospasm, oropharyngeal numbing, impairment or loss of gag reflex and/or impairment or loss of the tracheal aspiration reflex, and more typically in a patient suffering from or susceptible to oropharyngeal numbing, impairment or loss of gag reflex and/or impairment or loss of the tracheal aspiration reflex. The pharmaceutical composition or carcainium salt is particularly effective in such patients, and also due to the low systemic side effects associated with the invention.

Typically, said pharmaceutical composition or carcainium salt is for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, wherein said salt is (a) for use during a surgical or invasive procedure, or (b) for chronic use. A preferred surgical or invasive procedure where said carcainium salt can be used is bronchoscopy. Chronic use typically means administration of said carcainium salt twice a day or more, for example up to five times per day, or administration of said carcainium salt once a day or more over a period of one week or more, for example over a period of two weeks or more.

Typically, the pharmaceutical composition or carcainium salt is administered such that systemic exposure of carcainium salt following delivery to the patient as measured by peak plasma concentration is less than 800 ng/ml, more preferably less than 500 ng/ml, more preferably less than 100 ng/ml, and most preferably less than 70 ng/ml. The plasma concentration of carcainium salt can be measured by any suitable technique known to those skilled in the art, such as a liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay method.

The origin of the cough to be treated by the present invention is not particularly limited, and can include virtually any respiratory disorder, such as chronic obstructive pulmonary disease, asthma, tuberculosis, bronchitis, bronchiectasis, suppurative pulmonary disease, respiratory malignancies, allergy, cystic fibrosis, pulmonary fibrosis, respiratory tract inflammation, emphysema, pneumonia, lung cancer, lung neoplasia, sore throat, common cold, influenza, respiratory tract infection, bronchoconstriction, sarcoidosis, smoker's cough, chronic non-productive cough, neoplastic cough; cough due to gastroesophageal reflux, inhalation of irritants, smoke, smog, dust, presence of foreign bodies, air pollution or angiotension converting enzyme (ACE) inhibitor therapy, or acute or chronic cough resulting from or connected with a viral or bacterial infection of the upper airways; or intractable cough resulting from or connected with another underlying disease.

Typically, the underlying disease may be chronic obstructive pulmonary disease, asthma, tuberculosis, bronchitis, bronchiectasis, suppurative pulmonary disease, respiratory malignancies, allergy, cystic fibrosis, pulmonary fibrosis, respiratory tract inflammation, emphysema, pneumonia, lung cancer, lung neoplasia, soar throat, common cold, influenza, respiratory tract infection, bronchoconstriction, sarcoidosis, gastroesophageal reflux, smoker's

cough, chronic non-productive cough, neoplastic cough, or acute or chronic cough resulting from or connected with a viral or bacterial infection of the upper airways.

Alternatively, the origin of the cough to be treated by the present invention may be interstitial lung disease. In that instance, the cough, tussive attacks or tussive episodes result from interstitial lung disease. Interstitial lung diseases affect the interstitium, which is the tissue and space around the air sacs of the lungs, and in particular the alveolar epithelium, pulmonary capillary endothelium, basement membrane, perivascular and perilymphatic tissues.

Interstitial lung disease may be irritant-induced (for example by silica dust or asbestos) or drug induced (for example by antibiotics, chemotherapeutic drugs, antiarrhythmic agents, or statins). Interstitial lung disease may also arise from connective tissue diseases (such as systemic sclerosis, polymyositis, dermatomyositis, systemic lupus erythematosus or rheumatoid arthritis), from infection (such as atypical pneumonia, pneumocystis pneumonia (PCP), tuberculosis, chlamydia trachomatis or respiratory syncytial virus) or from malignancy (such a lymphangitic carcinomatosis). Interstitial lung disease may also be idiopathic, arising from for example sarcoidosis, idiopathic pulmonary fibrosis, Hamman-Rich syndrome or Antisynthetase Syndrome.

Typically, the pharmaceutical composition or carcainium salt is administered such that substantially whole dose of the drug is delivered to specific target areas, namely the trachea, carina and bronchi, while minimizing the deposition of the drug in other areas where it could cause undesirable local side effects or more easily enter the systemic circulation and cause undesirable side effects.

An aerosol, preferably delivered by pressurized metered dose inhaler, which has particles having a mass median aerodynamic diameter (MMAD) of from about 1μm to about 10μm, typically from about 3 μm to about 10 μm, is preferred. This allows effective delivery of the drug into the conducting and central airways. The carcainium salt is thus typically targeted to the conducting and central airways of the patient. The central airways are the region of the respiratory tract defined by trachea, carina and bronchi. The carina means the ridge separating the opening the right and left main bronchi at their junction with the trachea. Accordingly, the carcainium salt is typically delivered to the patient such that it does not cause bronchospasm, oropharyngeal numbing, impairment or loss of gag reflex, impairment or loss of the tracheal aspiration reflex or systemic exposure that leads to adverse side effects.

Efficacy of administration of carcainium salt is measured by the amount of the drug needed for cough abatement, by the frequency of administration needed to suppress tussive attacks or episodes, by the time necessary for delivery of the drug amount and by the percentage of the drug deposited in the specific target areas, namely in trachea, carina and bronchi as well as a lack of deposition in the other areas.

The magnitude of the therapeutic or prophylactic dose of carcainium salt required for the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient will depend upon the severity and nature of the condition being treated and the route of administration. The dose and the frequency of the dosing will also vary according to age, body weight and response of the individual patient. Typically, the daily dose is determined based on the weight of the patient. Preferably, the daily dose is 0.5 to 5 mg/kg, for example about 1.0 mg/kg, based on the weight of the patient.

Typically, the total daily dose of carcainium salt is from about 5 mg to about 300 mg. This may be delivered in a single dose or in repeated doses, for example up to five times a day, but is preferably delivered as a single dose. By daily dose it is meant the total quantity of compound of the invention administered to the patient in a day.

Typically the daily dose is a single metered nominal dose of from about 5 mg to about 300 mg. A metered nominal dose refers to the quantity of drug substance contained in the metering chamber of the delivery device and is normally expressed as quantity per actuation.

Upon actuation, the drug substance leaves the device and becomes available to the patient as a "delivered dose". The delivered dose is normally smaller than the metered nominal dose, due to the mechanics of the device. Thus, the delivered dose is the amount of the drug which is available at the mouth for inhalation. The delivered dose can be measured using standard techniques known to those skilled in the art. Typically, the delivered dose is from about 4.5 mg to about 275 mg.

Thus, the invention also provides a pressurised metered dose inhaler comprising pharmaceutical composition of the invention, which inhaler delivers an aerosol of carcainium salt and wherein the particles present in said aerosol have a mass median aerodynamic diameter (MMAD) of from about 1 μm to about 10μm and which inhaler is configured to deliver (a) a etered nominal dose of about 5 mg to about 300 mg carcainium salt, and/or (b) a delivered dose of about 4.5 mg to about 275 mg carcainium salt. Preferably the MMAD is from about 3 μm to

about 10 μm, more preferably from about 5 μm to about 10 μm, or from about 5.5 μm to about 10 μm or from about 6 μm to about 10 μm

Upon improvement of a patient's condition, a maintenance dose of carcainium salt may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. The patient may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

It will be understood, however, that the total daily usage of the carcainium salt will be decided by the attending physician within the scope of sound medical judgment. The specific dose for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

Any suitable route of administration may be employed to provide an effective dosage of the compounds of the present invention, although administration by inhalation is preferred, most preferably in aerosol form. Suitable forms of administration include, but are not limited to, inhalation (delivered by, e.g., metered dose inhaler, jet nebulizer, ultrasonic nebulizer, dry powder inhaler, etc.), nasal sprays, nebulization, oral administration such as via tablets, capsules, lozenges, syrups, sprays, suspensions, elixirs, gargles, and other liquid preparations, aerosol foams, parental administration, and sublingual administration. Administration by inhalation is preferred. Topical administration to the lung via inhalation, typically using a pressurized metered-dose inhaler is particularly preferred.

The pharmaceutical compositions of the present invention can include pharmaceutically acceptable carriers and other conventional additives, including aqueous based carriers, co-solvents such as ethyl alcohol, propylene glycol and glycerin, fillers, lubricants, wetting agents, flavoring agents, coloring agents, emulsifying, suspending or dispersing agents, suspending agents, etc. For aerosol delivery of the compounds of the present invention, pharmaceutically acceptable diluents, carriers, and/or propellants may be included in the compositions for use in

appropriate devices. These are prepared by procedures well known to those skilled in the art (see e.g., Medication Teaching Manual, 5th Ed., Bethesda, Md., American Society of Hospital Pharmacists, 1991).

The pharmaceutical compositions of the present invention may optionally include other known therapeutic agents, including decongestants such as pseudoephedrine HCl, phenylephrine HCl and ephedrine HCl, non-steroidal anti-inflammatory drugs such as acetaminophen, aspirin, phenacetin, ibuprofen and ketoprofen, expectorants such as glyceryl guaiacolate, terpin hydrate and ammonium chloride, antihistamines such as chlorpheniramine maleate, doxylamine succinate, brompheniramine maleate and diphenhydramine hydrochloride.

The pharmaceutical composition or carcainium salt of the invention can be administered in combination with (a) one or more additional anti-tussive agents and/or (b) one or more bronchodilators. Preferred additional anti-tussive agents are menthol or codeine. A preferred bronchodilator is N-{2-[(2E)-2-(mesitylimino)-9,10-dimethoxy-4-oxo-6,7-dihydro-2H-pyrimido[6,1-a]-isoquinolin-3(4H)-yl]ethyl}urea (which is known by the code RPL-554).

Accordingly, the present invention also provides a combination comprising a pharmaceutical composition or carcainium salt of the invention, and (a) one or more additional anti-tussive agents and/or (b) one or more bronchodilators. The combination is preferably for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient.

The invention further provides a pharmaceutical composition or carcainium salt of the invention for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, by co-administration with (a) one or more additional anti-tussive agents, and/or (b) one or more bronchodilators. Co-administration can be simultaneous, concurrent, separate or sequential.

The invention further provides (a) one or more additional anti-tussive agents and/or (b) one or more bronchodilators, for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient, by co-administration with a pharmaceutical composition or carcainium salt of the invention. Co-administration can be simultaneous, concurrent, separate or sequential.

The present invention further provides a product comprising a pharmaceutical composition or carcainium salt of the invention and (a) one or more additional anti-tussive agents and/or (b) one or more bronchodilators, as a combined preparation for simultaneous, concurrent,

separate or sequential use in the treatment and/or suppression of cough, tussive attacks or tussive episodes in a patient.

The salts of the invention can be prepared using the methods and procedures described herein, or using similar methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

For example, carcainium salts such as carcainium chloride can be synthesized as described in the Examples below. Alternatively, techniques such as those described in US 6,362,197 and Belgian Patent No. 614,154, which follows from Swedish Patent 1779/61, can be used. A conventional route of synthesis involves three steps and can be described (as in the aforementioned patent; see also T. Takahashi, J. Okada, M. Hori, A. Kato, K. Kanematsu, and Y. Yamamoto, J. Pharm. Soc. Japan 76, 1180-6 (1956)) as follows:

i) Chloroacetanilide

To a chilled solution of aniline (37.2 g, 0.40 mol) and potassium carbonate (66.4 g, 0.48 mol) in chloroform (200 ml) was added dropwise via cannula a solution of chloroacetylchloride (49.6 g, 0.44 mol) in chloroform (100 ml) and the reaction mixture was heated to 55 ^{o}C for 90 min. To the cooled reaction mixture was then added water (300 ml), the organic layer was collected and the aqueous layer was extracted twice more with chloroform (2 X100 ml). The combined organic layers were dried over sodium sulfate and evaporation of the solvent in vacuo provided the crude product. The product was purified via extraction through a Soxhlet apparatus with diethyl ether to provide 22 g of the desired chloroacetanilide. m.p. 133-135 ^{o}C.

ii) Dimethylaminoacetanilide

A mixture of chloroacetanilide (10.0 g, 59 mmol) in dimethylamine, 40% wt in water (100 ml) was refluxed for 4 hours. The cooled reaction mixture was partitioned between dichloromethane (100 ml) and 1M NaOH aqueous solution (100 ml). The aqueous layer was extracted twice more with dichloromethane (2 X100 ml), the combined organic layers were concentrated in vacuo to a

volume of approximately 100 ml and washed with water (2 X100 ml) in order to remove the remaining dimethylamine. The organic layer was collected, dried over sodium sulfate and the solvent evaporated in vacuo to provide 10.2 g (97% yield) of the pure dimethylaminoacetanilide.

iii) N,N-Bis-(phenylcarbamoylmethyl)dimethylammonium chloride (carcainium chloride)

A mixture of chloroacetanilide (10.1 g, 59.5 mmol), dimethylaminoacetanilide (10.7 g, 60 mmol) and potassium iodide, 99+% (0.1 g, 0.6 mmol) in dry xylene (30 ml) was refluxed for 1 hour and then allowed to stand overnight to ambient temperature. The solvent was decanted and the remaining gummy solid was triturated in diethyl ether in order to obtain a whitish powder. The resulting solid was collected and recrystallized in a mixture of ethanol and diethyl ether to provide 9.3 g (45% yield) of the desired ammonium salt. m.p. 177-178 ^{o}C.

The following Examples illustrate the invention.

EXAMPLES

Reference Example 1 - a clinical study in humans

The aim of the clinical study was to determine the clinical effectiveness and safety of carcainium chloride by the inhaled route in hospital in-patients with intractable, persistent cough due to interstitial lung disease.

A double blind, randomized, placebo-controlled, cross-over study design was used to assess the effect of carcainium chloride as an anti-tussive in patients with interstitial lung disease.

The study was constructed as an adaptive contingency trial. In such a trial the outcome of each “test” of the drug against placebo is scored as either a positive or negative result (i.e. a binary decision, rather than a quantitative measure). Based on the outcome of this “test”, the trial either continues or halts. Patients attended two study visits where they were randomised to receive either:

- carcainium chloride at a dose of 1.0 mg/kg on the first study visit followed by placebo (sodium chloride 0.9%) on the second visit; or

- placebo (sodium chloride 0.9%) on the first visit followed by carcainium chloride at 1.0 mg/kg on the second visit.

Assessment of each patient was based on the following criteria.

1. A physician's professional judgment of individual patient responses in a double blind crossover study with carcainium chloride and placebo in terms of anti-tussive action.

2. Each patient's subjective comfort using a visual analogue scale (VAS), pre- and post-treatment.

3. Each patient's coughs recorded in pre- and post-treatment periods for active and placebo treatments.

Preparation and administration of active and placebo samples

Carcainium chloride is a fine white dry powder and was provided in tightly closed vessels and stored in dark at room temperature upon receipt. The vehicle used for the dilution of carcainium chloride and for the placebo was 0.9 % NaCl injection.

Patients were then administered the active or placebo sample, according to the above schedule, as an aerosol generated using an ultrasonic nebulizer (DeVilbiss Ultraneb).

1. Physician Assessment

In 8 out of 8 trials, the two physicians acting in concert successfully identified the active treatment carcainium chloride. The outcome of this assessment or "test" after each study treatment administered was either a success in which an 'anti-tussive' effect was observed (i.e. positive) or a failure which was defined as no change from baseline or pro-tussive effect (i.e. negative). The clinical investigator decided on a positive or negative outcome for each "test" in

a blinded fashion. The statistical significance of this finding is <0.05 by contingency table, 0.05-0.01 by Chi square.

2. Patient comfort score

This was assessed on a 0-10 equal interval scale in the pre-drug and post-drug periods for active drug and placebo periods. A statistically significant improvement in patient well-being was observed with patients treated with carcainium chloride as compared to patients treated with placebo ($p = 0.0140$ when assessed by sum of signed ranks).

3. Number of coughs recorded electronically

The frequency of cough (number per unit time) was recorded using a semi-automated system which records cough epochs (sounds) that are counted by a qualified technician. There was a statistically significant treatment effect ($p = 0.0007$) associated with carcainium chloride as compared to placebo.

Summary of the results from the clinical study

Preliminary analysis of the clinical study results showed that carcainium chloride has marked anti-tussive activity which could be detected with subjective measures by two physicians when tested in a double blind randomized cross over contingency trial. All three measures of carcainium chloride's effectiveness revealed a statistically significant anti-tussive response. The patient comfort score (VAS) showed marked improvement in patient well-being as did the more objective measure involving the number of coughs recorded electronically.

Reference Example 2 - measurement of particle size distribution

Carcainium chloride aerosols corresponding to those used in the above clinical study were generated from the same drug product batch and using the same ultrasonic nebulizer (DeVilbiss Ultraneb). The particle size distributions of these carcainium chloride aerosols were measured and analyzed using a Malvern Spraytec with inhalation cell attachment system. Results from two replicate experiments showed an average value of about 5.38 μm for the Spraytec volume median diameter [Dv(50)].

It is generally accepted in the literature that laser diffraction techniques will agree with aerodynamic techniques when measuring spherical particles of unit or similar density, for example water or aqueous solution aerosols such as those generated above. Therefore the above average Dv(50) value of about 5.38 μm can be taken to correspond to an average mass median aerodynamic diameter (MMAD) value of about 5.38 μm for the particle size distribution of the carcainium chloride aerosol used in the above clinical study.

Reference Example 3 – measurement of carcainium chloride concentrations in human plasma

A liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay method was developed, qualified, and implemented for the quantitation of carcainium chloride levels in K_2EDTA human plasma samples collected from the human clinical study detailed above.

Initially, carcainium chloride was dissolved in deionized water to provide an initial standard or quality control (QC) stock solution at a concentration of 1000 μg/mL. Serial dilutions were carried out with deionized water to provide secondary stock solutions for subsequent preparation of plasma calibration standards or QC samples according to Table 1 below. The internal standard lidocaine was dissolved in deionized water to provide an initial stock solution at a concentration of 1000 μg/mL. The initial internal standard stock solution was then serially diluted with deionized water to provide a spiking stock solution concentration of 50 ng/mL.

Calibration Standards or QC Samples (ng/mL)	Stock Solutions Used (ng/mL)	Vol of Stock Solution (μL)	Vol of Human K_2EDTA Plasma (μL)	Final Conc. (ng/mL)
STD-500	STD-5000	10	100	500
STD-400	STD-4000	10	100	400
STD-100	STD-1000	10	100	100
STD-20	STD-200	10	100	20
STD-10	STD-100	10	100	10
STD-2.0	STD-20	10	100	2.0
STD-1.0	STD-10	10	100	1.0

STD-0.5	STD-5	10	100	0.5
QC-400	QC-4000	10	100	400
QC-80	QC-800	10	100	80
QC-1.5	QC-15	10	100	1.5

Table 1

A 10 µL aliquot of calibration standard stock solution or QC stock solution was transferred into individual 16x100 mm screw cap glass test tubes. For blank and blank with internal standard samples, 10 µL of deionized water was transferred instead. Human blank K_2EDTA plasma (100 µL) was then added to each tube. A 50 µL aliquot of of lidocaine internal standard spiking stock solution was transferred to each tube except for blank samples. A 50 µL aliquot of deionized water was added to blank samples. Samples were then vortex mixed.

A 100 µL aliquot of 1 M sodium hydroxide (NaOH) in deionized water was then added to each tube, followed by vortex-mixing. Methyl tert-butyl ether (3 mL) was then added to each tube followed by vortex-mixing for at least 20 seconds. The samples were then frozen at -80°C for at least 10 minutes. The top layer was transferred to 13x100 mm glass test tubes.

The supernatant was dried under a gentle stream of nitrogen to complete dryness using a Turbovap or under a gentle stream of air using an air dryer. Each sample was reconstituted with 100 µL of a 1:4 (v/v) mixture of 0.1% FA in MeOH:0.1% FA in deionized water. To facilitate reconstitution, the mixture was vortex-mixed for 1 minute followed by sonication for 5 minutes before being transferred to a 250 µL vial and capped. All vials were centrifuged at 5,000 rpm for 5 min and an aliquot of 25 µL was injected for LC/MS/MS analysis.

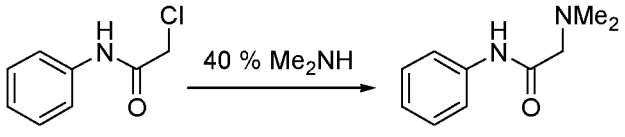
For human plasma samples 100- µL of sample was transferred into individual 16x100 mm screw cap glass test tubes. A 10 µL aliquot of deionized water was then transferred to each tube. A 50 µL aliquot of lidocaine internal standard solution was transferred to each tube and vortexed to mix. Test samples were then processed the same as calibration standards and quality control samples mentioned above.

The calibration curves established above were used to determine the concentration of carcainium chloride in plasma samples from patients who had been administered 1 mg/kg carcainium chloride. This was found to be in the range of 1-50 ng/ml. This a measure of systemic exposure to the drug, and the low levels of systemic exposure of the drug observed are

consistent with the general observation that no significant drug related adverse side effects were encountered in the human clinical study.

Example 1 – synthesis of carcainium salts

Step 1



C_8H_8ClNO
Mol. Wt.: 169.61

$C_{10}H_{14}N_2O$
Mol. Wt.: 178.23

2-Chloroacetanilide (460 g) and 40 % dimethylamine in water (4645 ml) were heated to reflux (max 102.4 °C) and stirred for 1.5 hours. HPLC analysis indicated 76.5 % stage 1 and 14.6 % 2-chloroacetanilide. Further 40 % dimethylamine in water (780 ml) was added and the reaction mixture was left to stir for 1 hours then allowed to cool to room temperature. HPLC analysis indicated 77.3 % stage 1 and 8.6 % 2-chloroacetanilide. The reaction mixture was heated back to 100 °C for 3 hours but no further conversion took place.

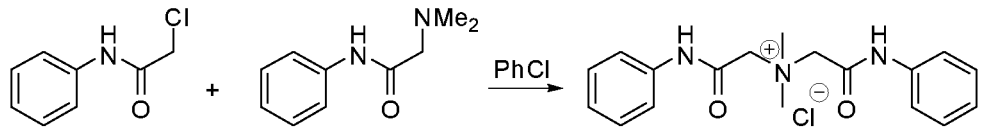
The reaction mixture was cooled to room temperature and DCM (2565 ml) was charged and stirred for 15 minutes. The layers were separated and the aqueous was extracted with DCM (1860 ml). The organic layers were combined and charged to the flask with water (4930 ml). The pH was adjusted to 1 by the addition of concentrated HCl (280 ml). The layers were separated and the aqueous layer was washed with DCM (1860 ml).

The aqueous layer was charged to the flask with DCM (4340 ml) and adjusted to pH8 by the addition of 50 % aq. NaOH (185 ml). The layers were separated and the aqueous layer was extracted with DCM (1860 ml). The organic layers were combined and washed with water (3285 ml), dried over $MgSO_4$ (660 g), filtered and concentrated to isolate a pale yellow oil.

The crude material was purified by silica gel chromatography (30 equivalents) and the product was eluted using ethyl acetate.

Yield = 293.6 g (60.7 %)

Step 2 – formation of carcainium chloride



C_8H_8ClNO
Mol. Wt.: 169.61

$C_{10}H_{14}N_2O$
Mol. Wt.: 178.23

$C_{18}H_{22}ClN_3O_2$
Mol. Wt.: 347.84

(a) Under N_2 was charged the product from Step 1 (85.0 g, 0.477 mol), 2-chloroacetanilide (78.8 g, 0.465 mol), KI (0.26 g) and chlorobenzene (1640 ml). The reaction mixture was heated to 100 °C and left to stir for 70 hours. HPLC analysis showed 3.2 % residual Step 1 product. The reaction mixture was cooled to room temperature and stirred for 4 hours. The resulting white precipitate was collected via vacuum filtration and washed with toluene. Yield = 146.3 g (90.5 %), HPLC analysis: 99.7 %, ^{1}H NMR showed no residual solvents.

(b) Under N_2 was charged the product from Step 1 (209.3 g), 2-chloroacetanilide (194.2 g), KI (0.64 g) and chlorobenzene (4040 ml). The reaction mixture was heated to 100 °C and left to stir for 48 hours. HPLC analysis showed 14.3 % residual Step 1 product. Due to no further progression in the reaction the reaction mixture was cooled to room temperature and stirred for 1 hour. The resulting white precipitate was collected via vacuum filtration to isolate a white solid. Yield = 359.2 g (88.0 %), HPLC analysis: 96.4 %.

(c) The crude material from step (b) (359.2 g) was combined with that from step (a) (146.3 g) and heated to 65 °C in ethanol to form a solution; then hot filtered to remove any particulates. The filtrate was charged with acetonitrile (6270 ml) and the solvents were distilled (6000 ml) until a temperature of 80-81 °C was achieved. The solution was slowly cooled to room temperature, where a solid precipitated at ~40 °C. The suspension was cooled further to 0 °C using an acetone/CO_2 bath and allowed to stir for 2 hours. The solid was collected *via* vacuum filtration, washed with acetonitrile (2 x 500 ml) and pulled dry on the filter for 2 hours. Yield = 373.2 g, HPLC analysis: 99.7 %.

(d) The liquors were concentrated to dryness and slurried in acetonitrile (160 ml) for 2 hours. The solid was collected *via* vacuum filtration and washed with acetonitrile (50 ml). Yield = 28.5 g, HPLC analysis: 98.8 %, ^{1}H NMR showed evidence of impurities.

(d) The solid was charged with ethanol (86 ml) and heated to form a solution. Acetonitrile (354 ml) was charged and the solvents were distilled until a temperature of 80-81 °C

was achieved. The solution was slowly cooled to room temperature, where a solid precipitated at ~40 °C. The solid was collected *via* vacuum filtration, washed with acetonitrile (2 x 10 ml) and pulled dry on the filter for 2 hours. Yield = 11.1 g, HPLC analysis: 99.6 %, ^{1}H NMR and XRPD confirmed formation of carcainium chloride.

Step 3 – formation of carcainium hydroxide

Anion exchange resin Amberlyst A-26 hydroxide form (28.2 g) was soaked in methanol (100 ml) for 10 minutes with swirling/rotation and filter; it was then rinsed with 2 x 100 ml methanol. The rinsed resin was suspended in methanol (470 ml) and carcainium chloride from step 2 was added as a solution in methanol (4.7 g, 94 ml). The mixture was rotated/gently agitated without grinding for 1.5 hours, with simultaneous testing of the solution above the resin for pH > 8.

When this pH was achieved, the solution was tested for residual chloride ions. A sample of the solution was mixed with water (deionised) and 17 % aqueous nitric acid (1-2 drops to pH 1). Silver nitrate standard solution (from Aldrich) was added dropwise. Formation of a white precipitate indicates that chloride ions are present in solution.

Once the solution tested negative for chloride ions, the resin was filtered and rinsed with 2 x 100 ml methanol and the liquors were reduced *in vacuo* at 35 °C, following which a solid of carcainium hydroxide formed. Yield of 80 %, HPLC purity 98.8 %.

Step 4 – formation of carcainium salts

Carcainium hydroxide (7 g, 6.56 g active, 0.02 mol) as a solution in methanol (240 ml) was stirred whilst a steady stream of the desired acid solution (1M, 1 equivalent, 20 ml), in methanol for organic acids (oxalic acid, maleic acid, fumaric acid or xinafoic acid) or 1:1 methanol/water for inorganic acids (sulfuric acid or hydrobromic acid), was charged. The mixture was stirred for 5 minutes and the liquors were pH tested. A pH of 4-5 was taken to indicate completion of the reaction. The solution was then evaporated to dryness, yielding a solid and/or a gum.

Each salt was then crystallised from water or another defined solvent between room temperature and 40 °C as described below.

Oxalate

Form conversion of the oxalate salt was undertaken by water maturation at 40°C (5.1 g in 25 ml for 18 hours). Recovery 4.5 g, 88.7 %. Chemical purity by HPLC: 100.0%. The ^{1}H NMR spectrum indicated salt formation, and the stoichiometry measured by ion-chromatography was 1:1.

Maleate

Form conversion of the maleate salt was undertaken by isopropyl alcohol (IPA) maturation at 40°C for 4 hours (3.76 g in 74 ml), followed by reduction *in vacuo* (reduced to 20 ml) and then maturation at 25°C for 2 hours. Recovery 100 % (3.76 g). Chemical purity by HPLC: 99.7%. The ^{1}H NMR spectrum indicated salt formation and 1:1 stoichiometry.

Bromide

Form conversion of the bromide salt was undertaken by water maturation at 40°C (7.76 g in 25 ml for 18 hours). Recovery 95 % (7.37 g). Chemical purity by HPLC: 100.0%. The ^{1}H NMR spectrum indicated salt formation and stoichiometry measured by ion-chromatography as 1:1.

Sulfate

Form conversion of the sulfate salt was undertaken by water maturation at 40°C (7.93 g in 25 ml for 18 hours. Recovery 87 % (6.92 g). Chemical purity by HPLC: 99.9%. The ^{1}H NMR spectrum indicated salt formation. Ion-chromatography revealed a 2:1 stoichiometry (bis-carcainium sulfate).

Fumarate

Form conversion of the fumarate salt was undertaken by IPA crystallisation. Dissolved 5.37g in 75 ml followed by cooling to ambient upon which crystallisation was apparent. Maturation at 40°C was employed for 18 hours. Recovery improved at 98 % (5.3 g). Chemical purity by HPLC: 100.0%. The 1H NMR spectrum indicated salt formation and 1:1 stoichiometry.

Xinafoate

The xinafoate was isolated by concentration *in vacuo*. Attempts to lyophilise led to oil and emulsion formation. Chemical purity by HPLC: 99.1%. The ^{1}H NMR spectrum indicated stoichiometry of 1:1. XRPD pattern of the 1-hydroxy-2-naphthoate did not contain any reflections and confirming that the xinafoate is amorphous.

Characterization of carcainium salts

The carcainium salts were characterized by X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The results are summarized in Table 2 below.

Salt	XRPD	DSC and TGA
Maleate	Crystalline (see Figure 1)	Onset of main endotherm at 145.2°C, peak at 148.97°C, ΔH: 51.34 J/g (see Figures 2 and 3).
Oxalate	Crystalline (see Figure 4)	Onset of main endotherm at 189.52°C, peak at 192.45°C, ΔH: 82.24J/g (see Figures 5 and 6).
Fumarate	Crystalline (see Figure 7)	Onset of main endotherm at 169.99°C, peak at 172.47°C, ΔH: 75.27 J/g (see Figures 8 and 9).
Sulfate	Crystalline (see Figure 10)	Onset of main endotherm at 162.50°C, peak at 168.68°C, ΔH: 49.27 J/g (see Figures 11 and 12).
Bromide	Crystalline (see Figure 13)	Onset of main endotherm at 176.22°C, peak at 178.79°C, ΔH: 70.10 J/g (see Figure 14 and 15).
Xinafoate	Amorphous	No thermal events as amorphous.

Table 2

Example 2 – 7 day stability of salts at 80 °C

A stability study was performed using carcainium salts prepared in Example 1 within a convection oven equilibrated to 80 °C. Approximately 4 mg of each salt was weighed into a small glass vial. This operation was replicated such that two x 4 mg samples of each were available for isolation and testing by HPLC without sampling, after 1 day and after 7 days.

The headspace in each vial was not relevant as the vessel was open to the oven (> 99 % airspace above sample within the confines of the glass). The oven was open to ambient via a bleed valve. Samples were capped prior to submission for analysis by HPLC and cooled to ambient. All salts remained visually unimpaired. Purities by HPLC are shown in Table 3 below.

Salt	Initial purity (%)	Purity after 1 day (%)	Purity after 7 days (%)
Chloride	99.95	99.96	99.96
Maleate	99.70	99.67	99.67
Oxalate	99.97	99.96	99.95
Fumarate	99.96	99.86	99.96
Sulfate	99.92	99.94	99.93
Bromide	99.98	99.97	99.99
Xinafoate	99.07	95.69	92.55

The table above shows that the salts forms have good stability at elevated temperature.

Table 3

Example 3 – milling and analysis of milled carcainium salts

Jet-milling

The carcainium maleate, oxalate, fumarate, sulfate and bromide salts prepared in Example 1 where milled. A Sturtevant jet-mill fitted with a vibratory type feeder and bag filter for product collection (S/N 25208715) was used for the jet-milling process. The jet-mill was driven by compressed nitrogen supplied at 7 bar. Venturi and grind pressures were set at 90 psi and 60 psi (50 psi for sulfate), respectively, and each candidate salt was passed through the jet-mill once only.

Scanning Electron Microscopy (SEM)

A small quantity of each sample was deposited on a sticky carbon tab on a SEM stub and gold coated using an Agar sputter coater. The samples were then imaged by Scanning Electron

Microscopy using a LEO1430 VP SEM. The observations are summarised in Table 4 below and confirm that the milling process was successful.

Salt	Particle size after milling
Chloride	Typically 1-2 μm, very few particles greater than 5 μm.
Maleate	Typically 1-2 μm, very few particles greater than 5 μm.
Oxalate	Typically 1-2 μm, very few particles greater than 5 μm.
Fumarate	Typically less than 5 μm.
Sulfate	Typically 1-2 μm, very few particles greater than 5 μm.
Bromide	Typically 1-2 μm, very few particles greater than 5 μm.
Xinafoate	Typically 1-2 μm, very few particles greater than 5 μm.

Table 4

Laser diffraction

The salts were measured by laser diffraction fitted with a dry powder dispersal unit (Malvern Mastersizer 2000 & Sciroco 2000). The results are provided in Table 5 below.

Carcainium salt	d(0.1) (μm)	d(0.5) (μm)	d(0.9) (μm)	Obscuration (%)
Carcainium chloride	0.604	1.391	2.919	1.68
Carcainium bromide	0.592	1.410	3.051	2.49
Carcainium fumarate	1.157	2.345	4.618	1.23
Carcainium maleate	0.820	1.928	4.119	1.37
Carcainium xinafoate	0.712	1.710	3.705	1.59
Carcainium oxalate	0.711	1.669	4.336	1.26
Carcainium sulfate	1.053	2.340	4.919	0.95

Table 5

The result in Table 5 demonstrate that over 90% of the milled material had a particle size of less than 5 μm for all salt forms.

X-Ray Powder Diffraction (XRPD)

Powder X-ray diffraction was performed using a Bruker D8 Advance instrument in reflection geometry using Cu $K_{\alpha 1}$ radiation with an incident beam monochromator. The instrument was calibrated against a NIST standard of Corundum (NIST 1976). The samples were analysed as-received on a zerobackground Silicon sample holder, which was spun during analysis.

The xinafoate was not analysed as it is amorphous. The X-ray diffraction patterns for the unmilled salts were compared to the milled salts. In all cases, peaks were observed in the same places for the unmilled salts as the milled salts, indicating that there is no change in the crystalline phase caused by milling.

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA)

DSC analysis was performed on a TA Instruments Q2000 equipped with a Tzero cell purged at a constant purge flow rate of 50 ml/minute with dry Nitrogen, and with a RCS 90 cooling system. The instrument was calibrated using Indium as a standard. Between 3 and 5 mg of the samples were weighed into a TAI Tzero Aluminium pan with a pierced lid placed on top prior to being heated from -20 °C to 300 °C at 10 °C per minute, unless otherwise specified in the figure caption.

TGA was performed on a TA Instruments Q5000 equipped with an ultrasensitive balance and an infrared furnace purged with dry Nitrogen at a constant flow rate of 10 mL/minute for the reference and 25 mL/minute for the sample. The weight calibration was performed using a certified 100 mg calibration weight. The temperature calibration was performed using Alumel® and Nickel standards. Approximately 7 mg of the samples were weighed into TAI 100 μL Platinum sample pan/holders prior to being heated from ambient temperature to 300 °C at a rate of 10 °C per minute.

DSC and TGA results observed for the milled samples of chloride, maleate, oxalate, fumarate, sulfate and xinafoate were very similar to those observed for the unmilled samples,

indicating that milling does not cause a change in the crystalline phase. Changes were observed from the bromide salt, indicating that milling may causes changes in structure for this salt.

Example 4 – assessment of suitability of salts for use in pressurized metered dose inhalers

Clear pressurized metered dose inhaler (pMDI) canisters were filled with 24 mg samples of the milled salts from Example 4 were filled into to which was added one of the following 9 pMDI solvent mixtures.

1. HFA134a
2. HFA134a, 5% ethanol & 0.1% tween
3. HFA134a , 10% ethanol & 0.1 % tween
4. HFA134a/HFA227 1:1
5. HFA134a/HFA227 1:1, 5% ethanol & 0.1% tween
6. HFA134a/HFA227 1:1, 10% ethanol & 0.1 % tween
7. HFA227
8. HFA227, 5% ethanol & 0.1% tween
9. HFA227, 10% ethanol & 0.1 % tween

Formulations were visually assessed for dispersion, flocculation type, sedimentation rate, and sedimentation height. Based on this visual assessment, the rankings for suitability for use in pMDIs set out in Table 6 below were determined.

Ranking for potential for use in suspension pMDIs	**HFA134a solutions**	**HFA134a/HFA227 1:1 solutions**	**HFA227 solutions**
1st	Bromide	Xinafoate	Xinafoate
2nd	Sulfate	Bromide	Bromide
3rd	Xinafoate	Sulfate	Sulfate
4th	Chloride	Chloride	Chloride
5th	Fumarate	Fumarate	Fumarate
6th	Maleate	Maleate	Maleate

7th	Oxalate	Oxalate	Oxalate

Table 6

The fumarate, maleate and oxalate salt forms were not suitable for use in suspension pMDIs, due to aggregation of the salt, formation of a poor dispersion and rapid sedimentation.

The xinafoate, bromide, sulfate and chloride salts were considered suitable for use in suspension pMDIs. The bromide, sulfate and chloride salts were further tested for aerosol performance. In particular, the bromide, sulfate and chloride salts were tested for aersolisation quality on a next generation impactor. The fine particle fraction (FPF) and doses (salt) measured our set out in Table 7 below.

Salt	**Fine particle fraction % <5µm**	**Fine particle dose (µg)**
Sulfate	19.45	21.51
Chloride	13.20	16.22
Bromide	11.55	14.48

Table 7

The results in the table above demonstrate that the sulfate, chloride and bromide salts provide a good fine particle fraction when used in suspension pMDIs.

The solubility of the salts was measured in HFA134a with 10% ethanol and 0.1% Tween. Samples were extracted from vials under pressure and filtered through 0.22µm filters. The results are set out in Table 8 below.

Salt	**Solubility in HFA134a/EtOH/Tween [90:10:0.1] (µg/g)***	**Estimated solubilised carcainium base (µg/g) per 50 µL actuation**
Xinafoate	239.34	18.40
Maleate	202.04	12.81

Chloride	131.91	7.87
Bromide	38.96	2.43
Sulfate	8.80	0.55
Fumarate	0.03	0.00
Oxalate	Below limit of detection	0.00

* expressed as base

Table 8

The results in the table above demonstrate that the xinafoate, maleate and chloride are particularly suitable for use in solution pMDIs.

Example 5 – anti-tussive effect of carcainium salts

Detection of cough

Conscious male guinea pigs (350-600g) were placed in individual perspex cylindrical exposure chambers with airflow of 1.5 L/minute and allowed to acclimatize. After acclimatization and one minute of baseline recording, cough responses were induced by exposing animals to citric acid aerosol (0.3 M) generated by an ultrasonic nebuliser at a nebulisation rate of 0.1 ml/minute for 5 minutes (EMKA, France).

Coughs were counted throughout the 5 minute citric acid exposure and for a further 10 minutes. Individual coughs were detected in three ways: 1) via a pressure transducer attached to the exposure chamber amplified and recorded using the IOX (version 1.7.0) data acquisition system, 2) via a microphone placed inside the chamber amplified and recorded using IOX, and 3) via visual observation of the animal.

Coughs were counted by a trained observer and were distinguished from responses by the characteristic changes in pressure in the chamber and by visual observation. Animals were removed from the exposure chamber and allowed to recover before being returned to the animal house. Animals were given at least 96 hours to recover between exposures to citric acid and could be challenged up to 4 times.

Drug exposure

Animals (263 -342 g) were placed in custom built perspex exposure chamber with 4 arms. After acclimatisation, animals were exposed to nebulised (Devilbiss 99) solutions of vehicle (saline), carcainium salt (10 mg/mL). Animals were then removed and placed in the cough chambers. The time interval between post aerosol exposure and commencement of citric acid (12.6 g in 200 ml; 0.3 M) challenge to induce cough was between +25, +31, +37 and + 43 min post saline or drug challenge.

Experimental Design

A randomised cross-over experimental design was employed to assess the efficacy of carcainium salts in a model of citric acid induced cough. Animals were first screened and then randomized to receive either saline (0.9%), carcainium salt (10 mg/mL) prior to the measurement of cough.

The doses of each salt employed in this study were based calculated as set out in Table 9.

Salt form	**Theoretical molecular weight of salt (g/mol)**	**Molar concentration (moles/L) of "carcainium" in a 10mg/ml solution of salt**	**No of mgs of salt per ml required to achieve the same molar concentration of "carcainium" as in a 10mg/ml solution of carcainium chloride**
Carcainium chloride	347.84	0.0285	10.0
Carcainium bromide	392.29	0.0255	11.3
Carcainium sulfate	360.42	0.0277	10.4
Carcainium maleate	427.45	0.0234	12.3
Carcainium xinafoate	499.56	0.0200	14.4

Table 9

Solubility in saline solution

The carcainium salts were dissolved in 0.9 % saline. The xinafoate was relatively insoluble in 0.9 % saline, consequently, solutions were prepared in 4.7 % solutol (1.40 g solutol

in 30 mL of 0.9 % saline) for nebulisation. All remaining salts were soluble in 0.9 % saline at 1 and 10 mg/mL.

26-30ml of solutions, prepared in 0.9% saline, was nebulised over the period of 20 min whereas 18-25ml of solutions, prepared in 4.7% solutol in 0.9% saline, was nebulised over the period of 20 min. The pH of each solution is set out in Table 10 below.

Carcainium salt	**pH**
Carcainium chloride	4.05
Carcainium bromide	4.21
Carcainium sulfate	1.75
Carcainium maleate	4.02
Carcainium xinafoate	5.51

Table 10

Behavioural observations

Guinea-pigs were challenged with different salts of carcainium chloride in a 4 arm exposure chamber for 20 minutes. None of the carcainium salts caused any change in respiratory rate, breathing difficult or tussive responses in 8/8 animals exposed to 10 mg/mL of each salt.

This was a surprising observation, since the all of the salt solutions were acidic (see Table 10) and would have been expected to cause lung irritation on that basis.

In contrast, changes in breathing pattern and tussive responses was observed in 4/4 animals exposed to citric acid (12.6 g in 200 mL; 0. 3 M) in the exposure chamber.

Effect of carcainium salts on tussive response to citric acid

The mean (95 % CI) of cough in response to citric acid (0.3 M) at screening was 29 (26 – 32) in 55 animals. Carcainium salts caused a 40 -60 % inhibition of the cough response at a dose of 10 mg/mL. The results are depicted in Figure 16.

CLAIMS

1. A liquid pharmaceutical composition for use in a pressurized metered dose inhaler (pMDI), which composition comprises a pharmaceutically acceptable addition salt of (i) carcainium, and (ii) sulfuric acid, hydrochloric acid, hydrobromic acid, maleic acid or 1-hydroxy-2-naphthoic acid (xinafoic acid).

2. A pharmaceutical composition according to claim 1, which is a suspension of the salt.

3. A pharmaceutical composition according to claim 2, wherein the salt is carcainium sulfate, carcainium chloride, carcainium bromide or carcainium 1-hydroxy-2-naphthoate (xinafoate).

4. A pharmaceutical composition according to claim 1, which is a solution of the salt.

5. A pharmaceutical composition according to claim 4, wherein the salt is carcainium 1-hydroxy-2-naphthoate (xinafoate), carcainium maleate or carcainium chloride.

6. A pharmaceutical composition according to any one of the preceding claims, which comprises a hydrofluoroalkane propellant.

7. A pharmaceutical composition according to claim 6, where in the hydrofluoroalkane propellant is 1,1,1,2,-tetrafluoroethane, or 1,1,1,2,3,3,3-heptafluoropropane or a mixtures thereof.

8. A pressurized metered dose inhaler comprising a pharmaceutical composition as defined in any one of the preceding claims.

9. A pharmaceutically acceptable addition salt of (i) carcainium, and (ii) sulfuric acid, hydrobromic acid, maleic acid or 1-hydroxy-2-naphthoic acid (xinafoic acid).

10. A salt according to claim 9, which is carcainium sulfate.

11. A salt according to claim 9, which is carcainium bromide.

12. A salt according to claim 9, which is carcainium maleate.

13. A salt according to claim 9 which is carcainium 1-hydroxy-2-naphthoate (xinafoate).

14. A pharmaceutical composition as defined in any one of claims 1 to 7 or carcainium salt as defined in any one of claims 9 to 13, for use in the treatment of the human or animal body.

15 A pharmaceutical composition as defined in any one of claims 1 to 7 or carcainium salt as defined in any one of claims 9 to 13, for use in the treatment and/or suppression of cough, tussive attacks or tussive episodes.

16. A pharmaceutical composition or carcainium salt for use as defined in claim 15, wherein the cough, tussive attacks or tussive episodes result from interstitial lung disease.

17. Use of a pharmaceutical composition as defined in any one of claims 1 to 7 or a carcainium salt as defined in any one of claims 9 to 13 in the manufacture of a medicament for the treatment and/or suppression of cough, tussive attacks or tussive episodes as defined in claim 15 or 16.

18. A method of treatment and/or suppression of cough, tussive attacks or tussive episodes as defined in claim 15 or 16 in a patient, which method comprises administering to said patient a therapeutically effective amount of a pharmaceutical composition as defined in any one of claims 1 to 7 or a carcainium salt as defined in any one of claims 9 to 13.

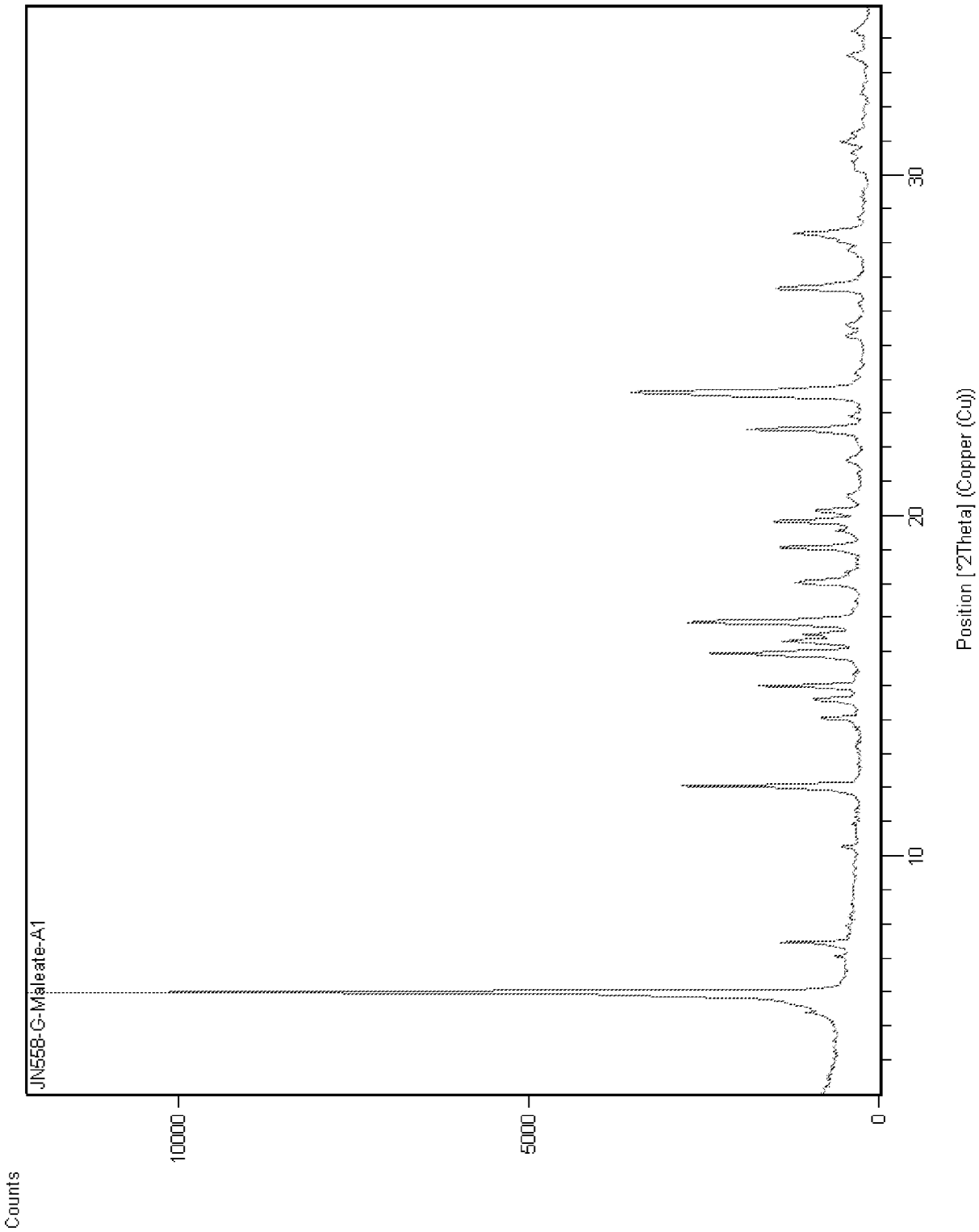

JN558-G-Maleate-A1
Counts
10000
5000
0
10
20
30
Position [°2Theta] (Copper (Cu))

Figure 1

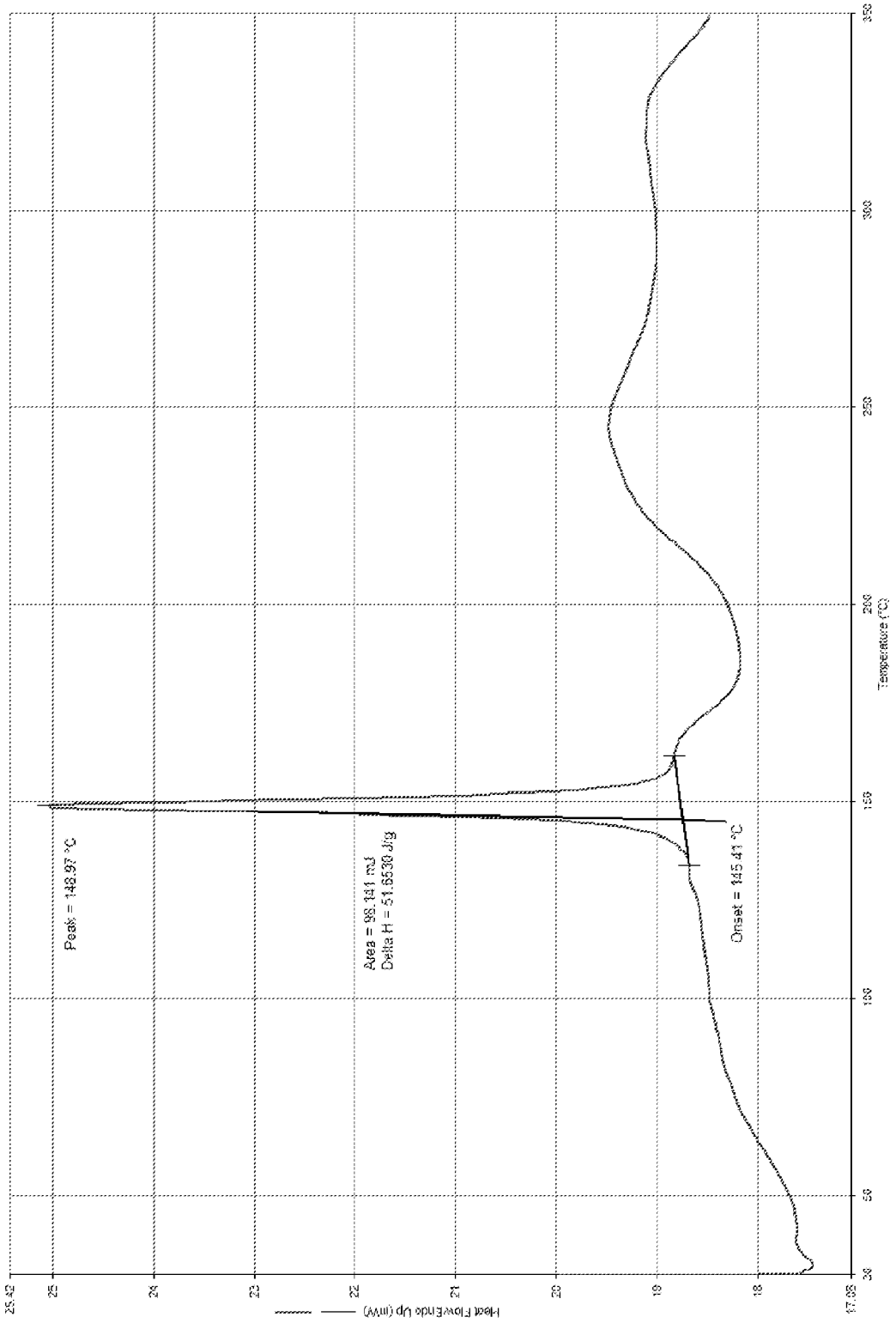

Peak = 148.97 °C
Area = 98.141 mJ
Delta H = 51.6539 J/g
Onset = 145.41 °C
Heat Flow Endo Up (mW)
Temperature (°C)
25.42
25
24
23
22
21
20
19
18
17.08
38
58
108
158
208
258
308
358

Figure 2

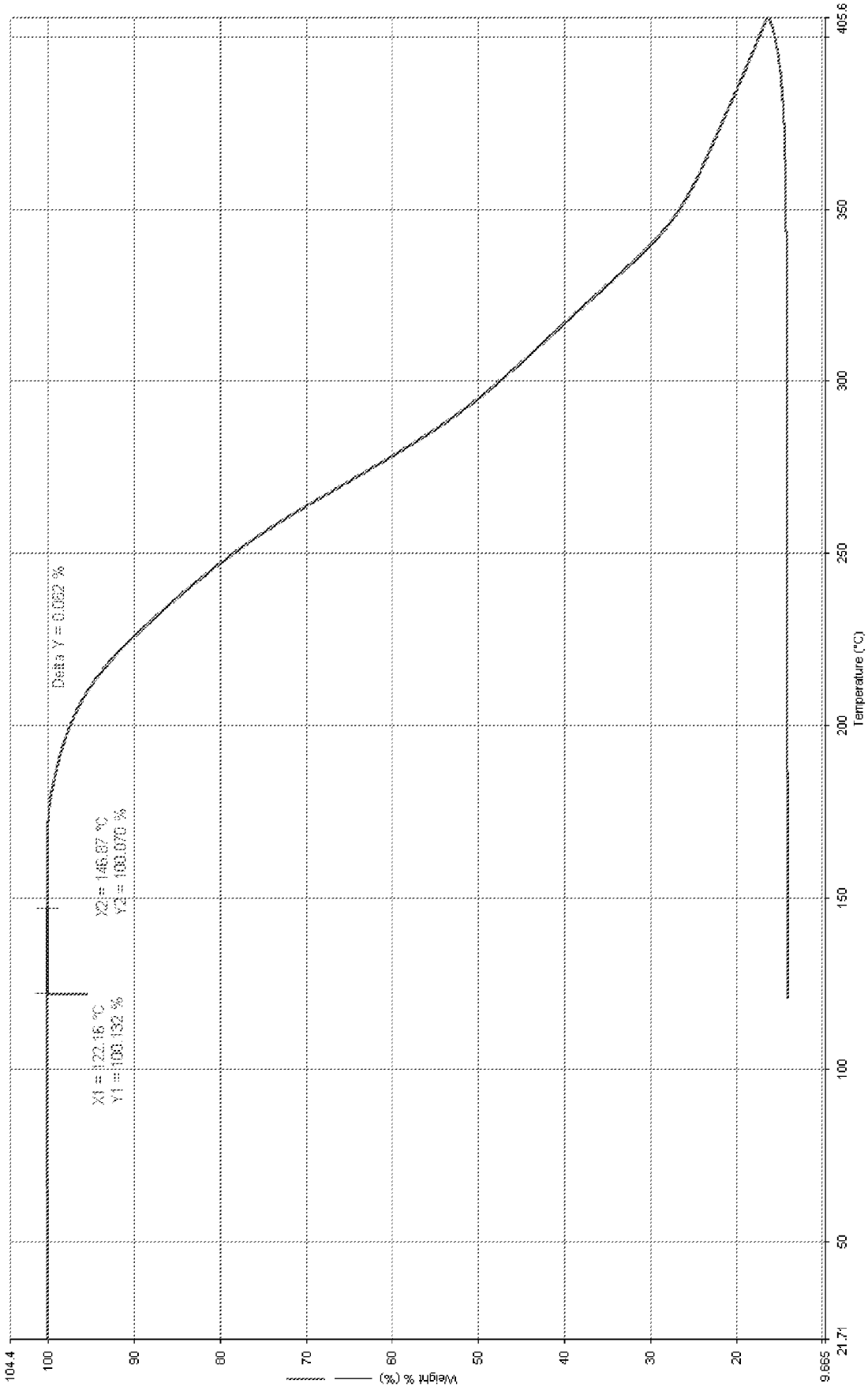

Delta Y = 0.082 %
X2= 146.87 °C
Y2= 100.070 %
X1 = 122.16 °C
Y1 = 108.132 %
Weight % (%)
Temperature (°C)
104.4
100
90
80
70
60
50
40
30
20
9.665
21.71
50
100
150
200
250
300
350
405.6

Figure 3

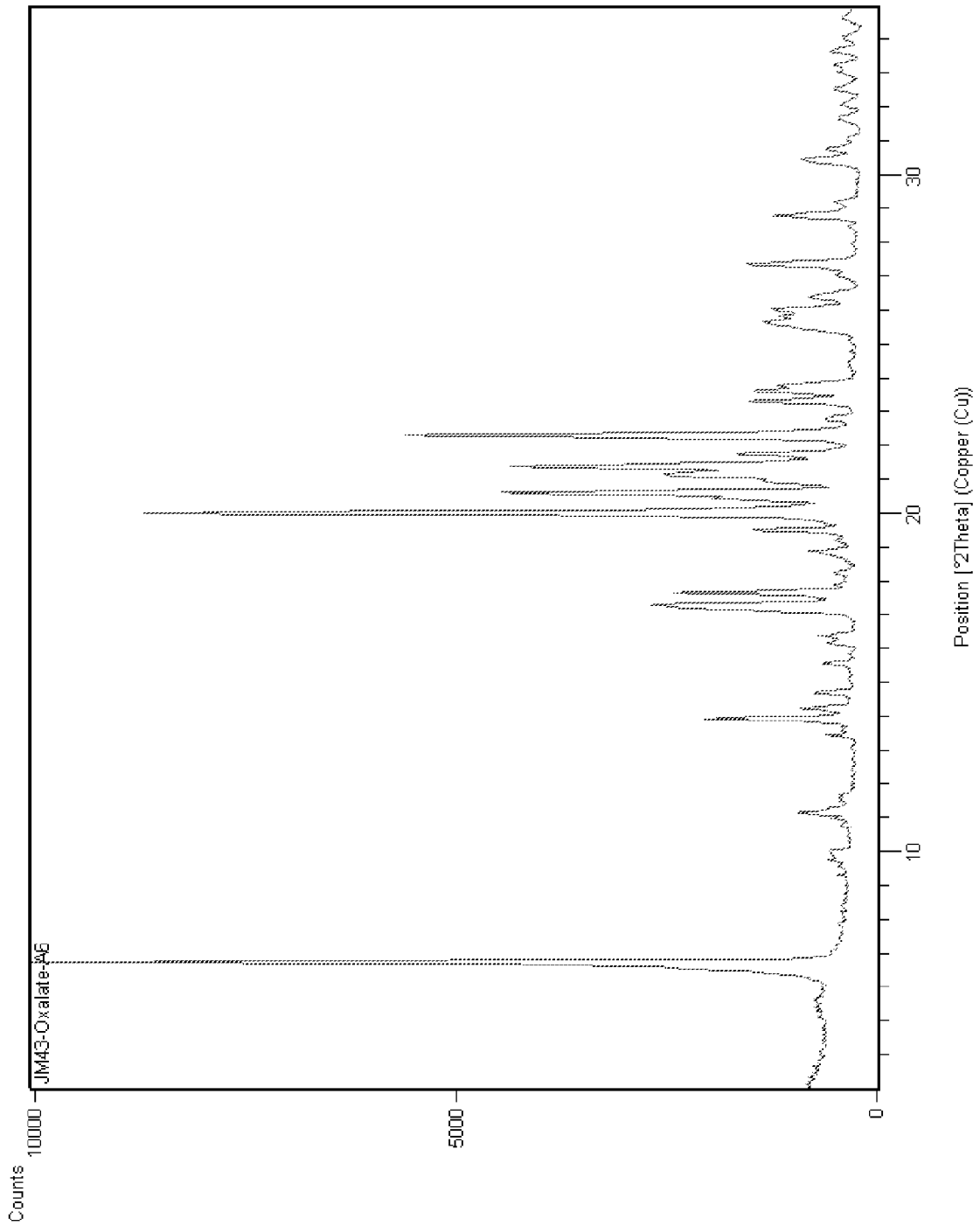

JM43-Oxalate-A6
Counts
10000
5000
0
10
20
30
Position [°2Theta] (Copper (Cu))

Figure 4

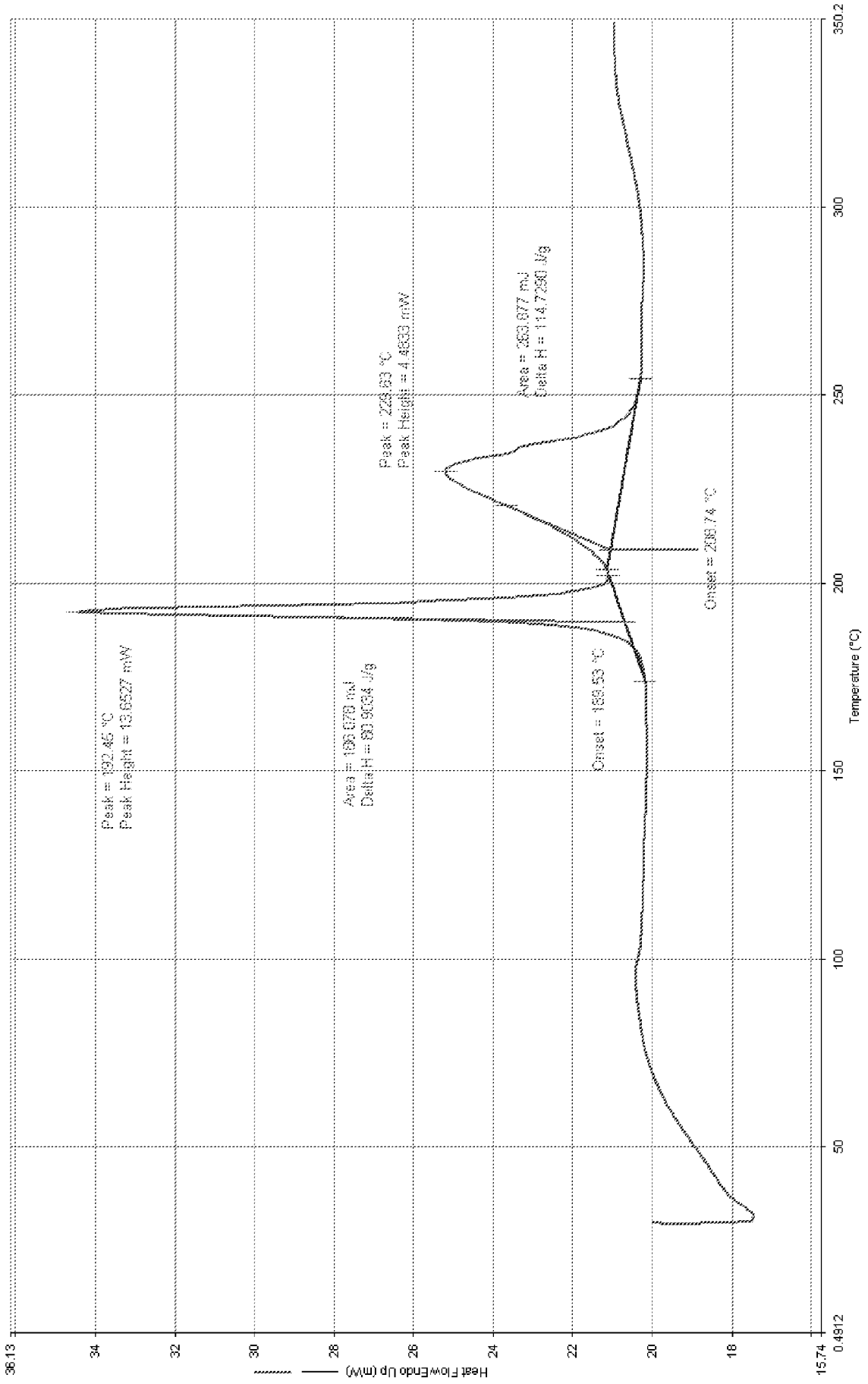

Peak = 192.46 °C
Peak Height = 13.6527 mW
Area = 196.676 mJ
Delta H = 80.9034 J/g
Onset = 189.53 °C
Peak = 229.83 °C
Peak Height = 4.4833 mW
Area = 353.677 mJ
Delta H = 114.7290 J/g
Onset = 206.74 °C
Heat FlowEndo Up (mW)
Temperature (°C)
36.13
34
32
30
28
26
24
22
20
18
15.74
0.4912
50
100
150
200
250
300
350.2

Figure 5

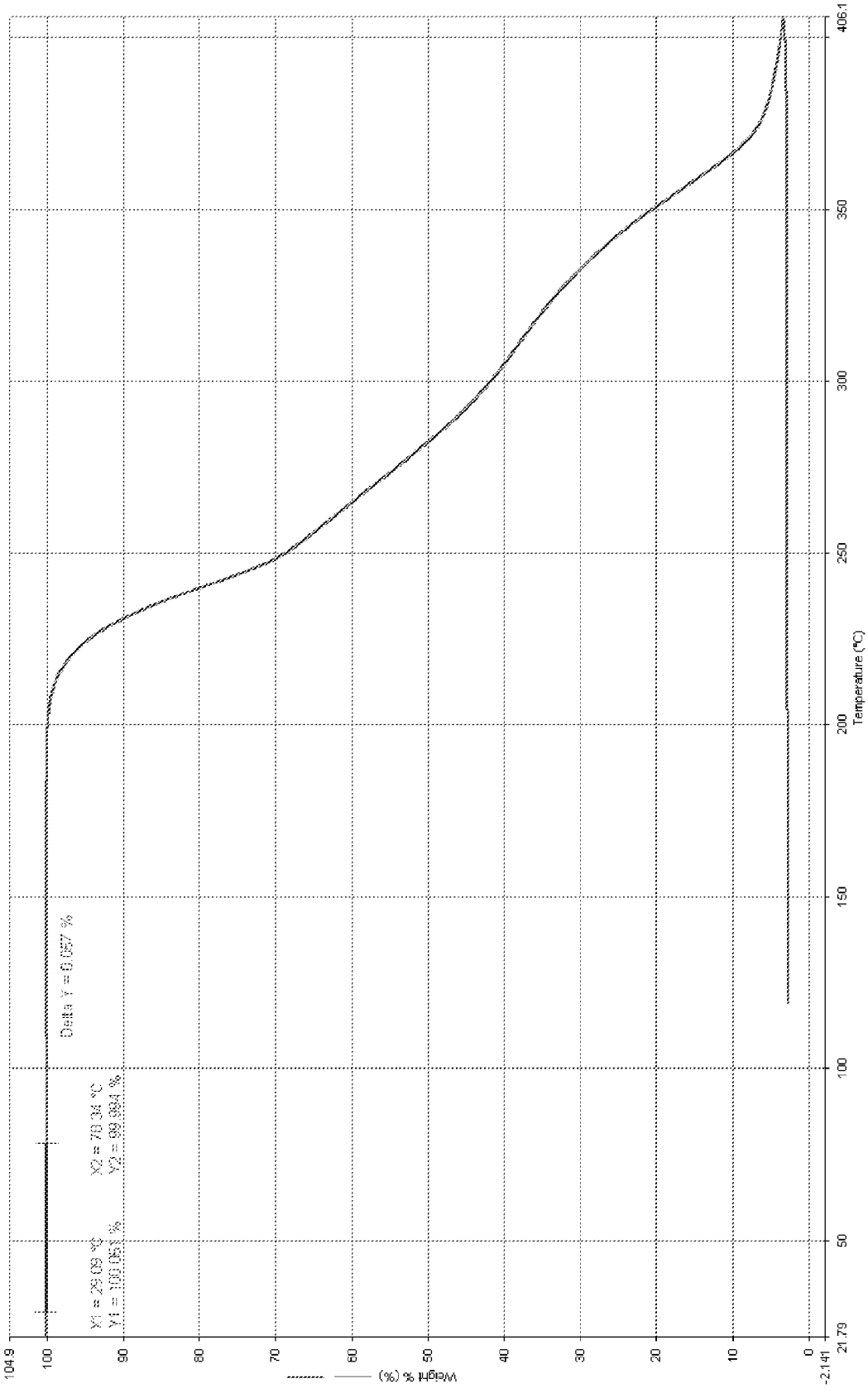

Weight % (%)
Temperature (°C)
Delta Y = 0.057 %
X1 = 25.09 °C
Y1 = 100.051 %
X2 = 78.34 °C
Y2 = 99.994 %
104.9
100
90
80
70
60
50
40
30
20
10
0
-2.141
21.79
50
100
150
200
250
300
350
406.1

Figure 6

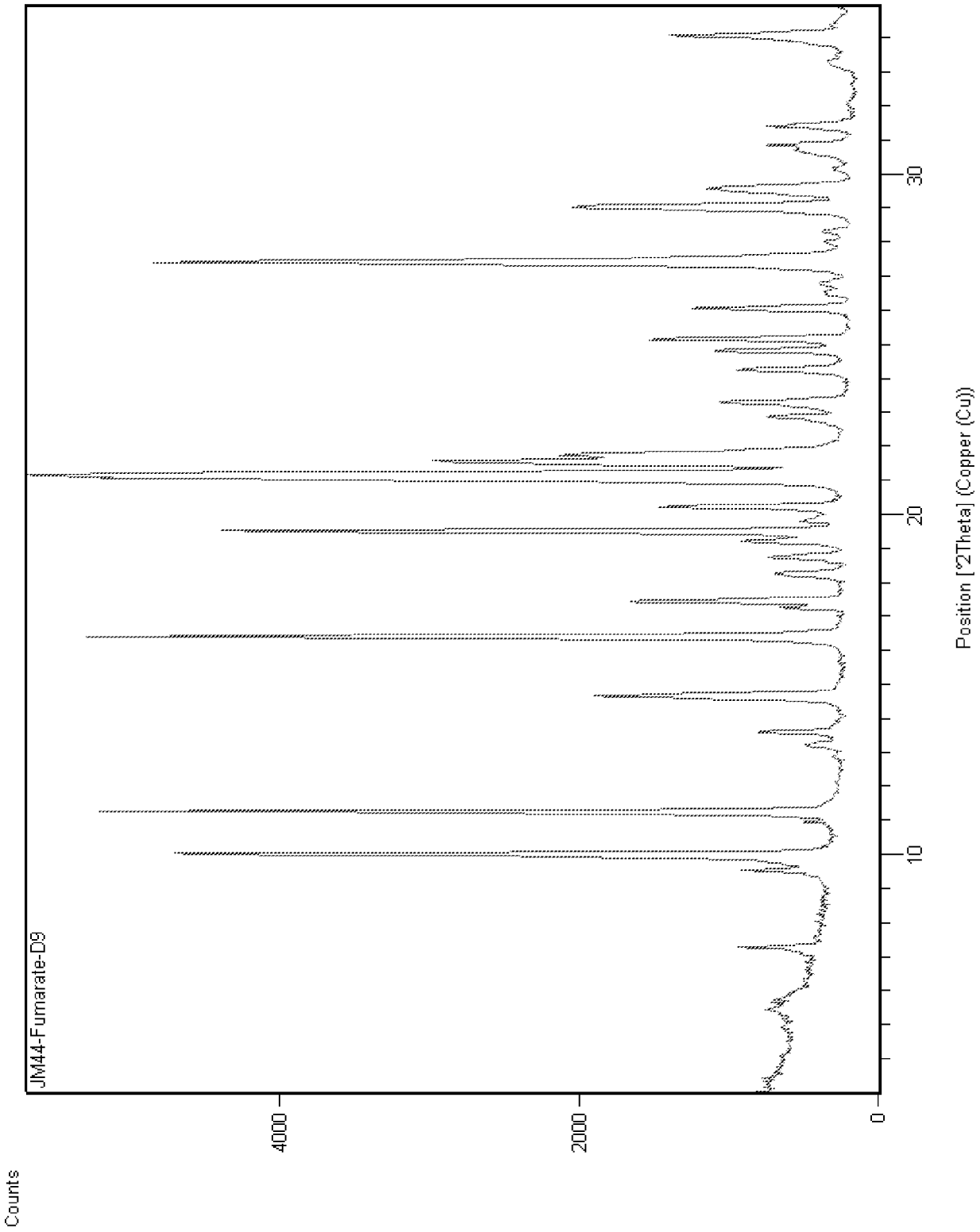

JM44-Fumarate-D9
Counts
4000
2000
0
10
20
30
Position [°2Theta] (Copper (Cu))

Figure 7

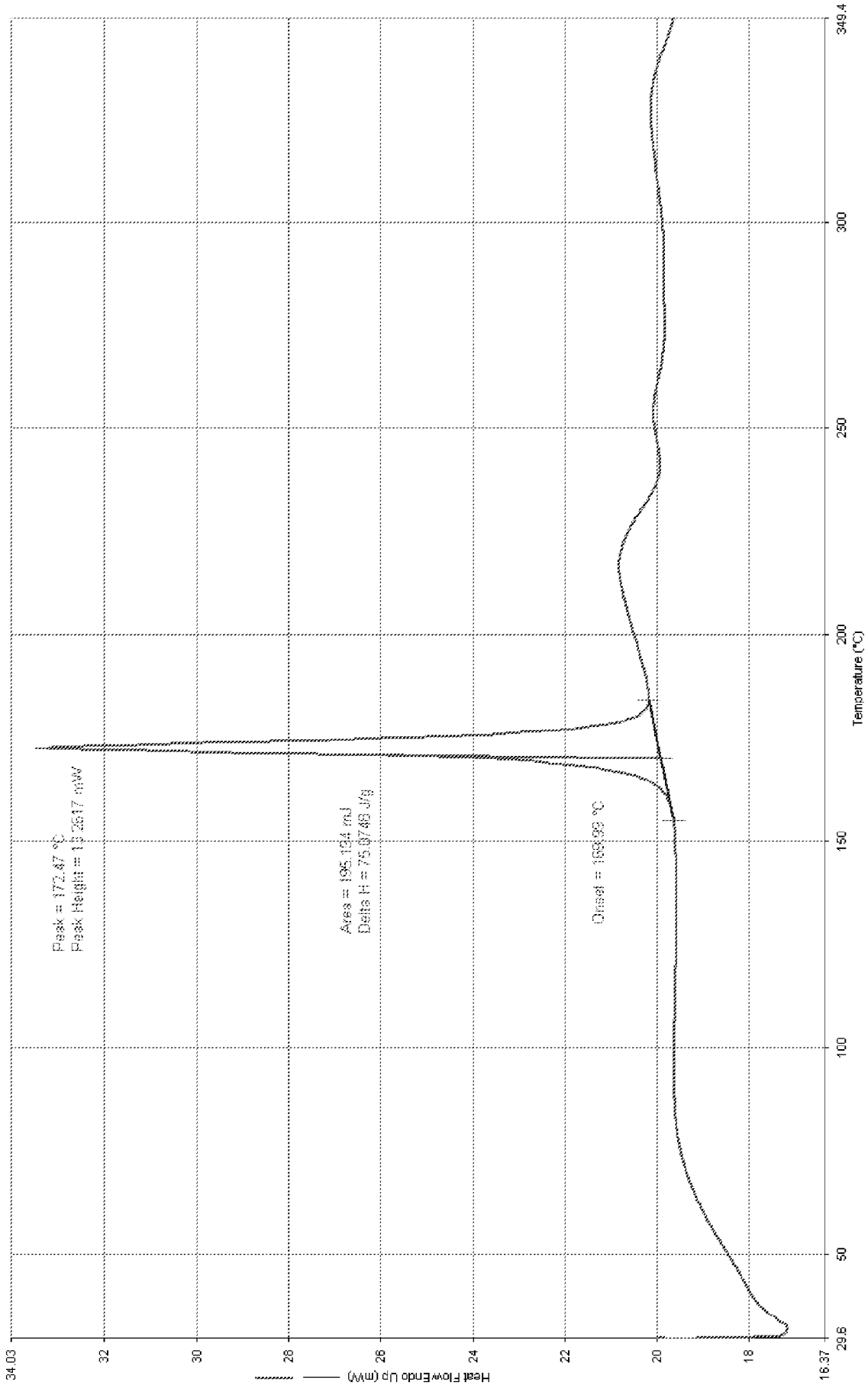

Peak = 172.87 °C
Peak Height = 13.2517 mW
Area = 195.194 mJ
Delta H = 75.0749 J/g
Onset = 169.99 °C
Heat FlowEndo Up (mW)
Temperature (°C)
34.03
32
30
28
26
24
22
20
18
16.37
29.6
50
100
150
200
250
300
349.4

Figure 8

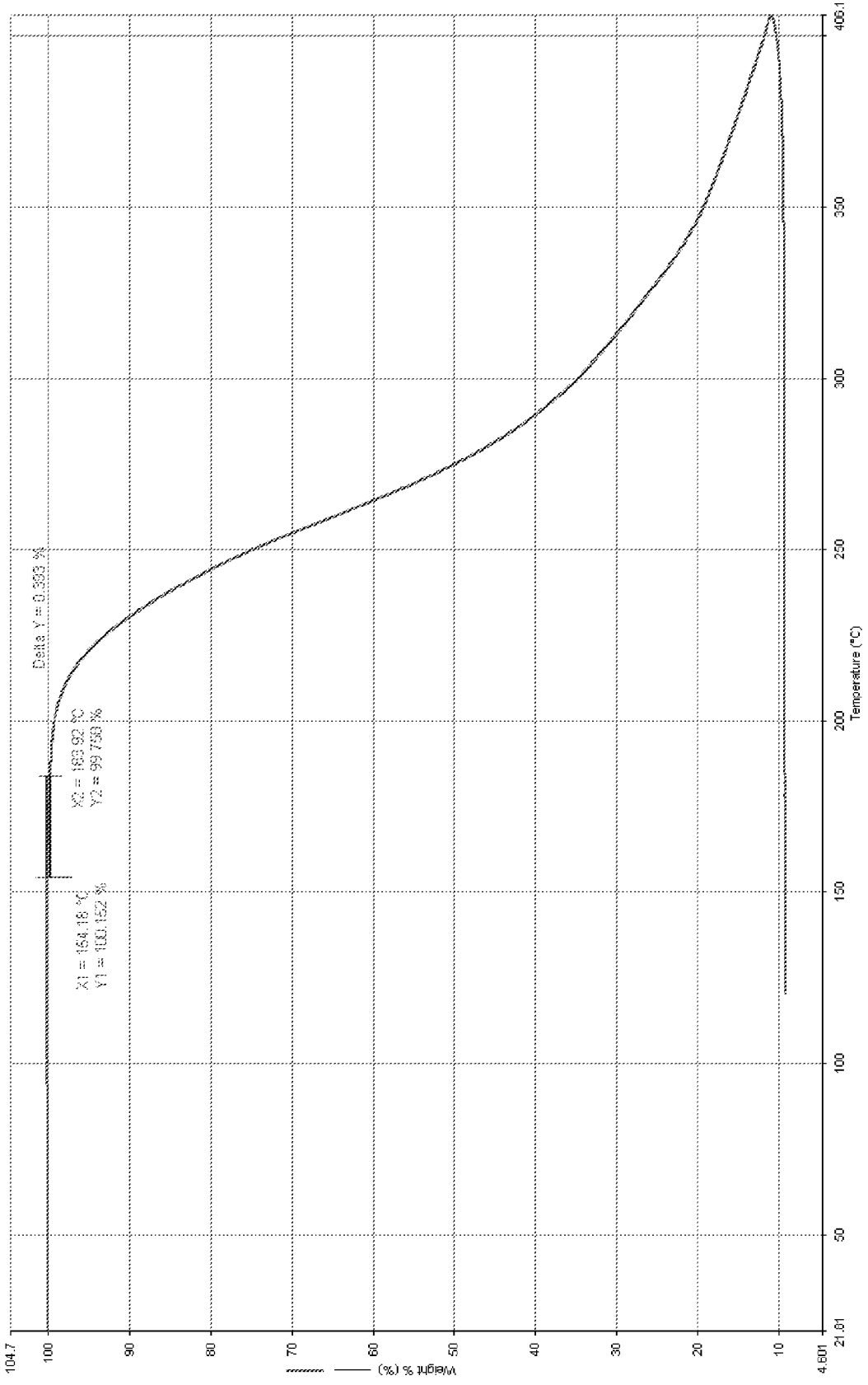

Delta Y = 0.393 %
X2 = 183.92 °C
Y2 = 99.759 %
X1 = 164.18 °C
Y1 = 100.152 %
Weight % (%)
Temperature (°C)
104.7
100
90
80
70
60
50
40
30
20
10
4.601
21.01
50
100
150
200
250
300
350
406.1

Figure 9

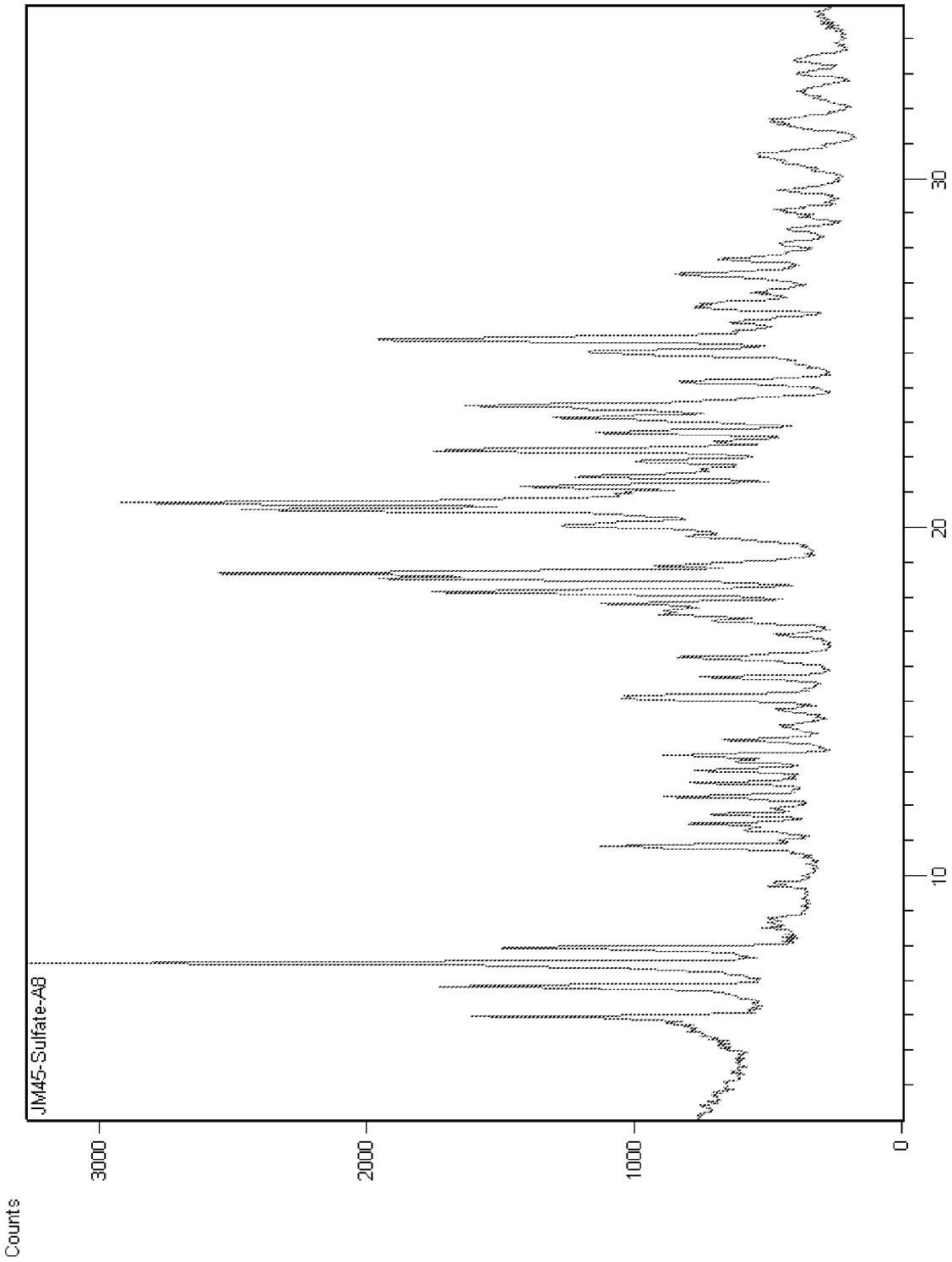

JM45-Sulfate-A8
Counts
3000
2000
1000
0
10
20
30
Position [°2Theta] (Copper (Cu))

Figure 10

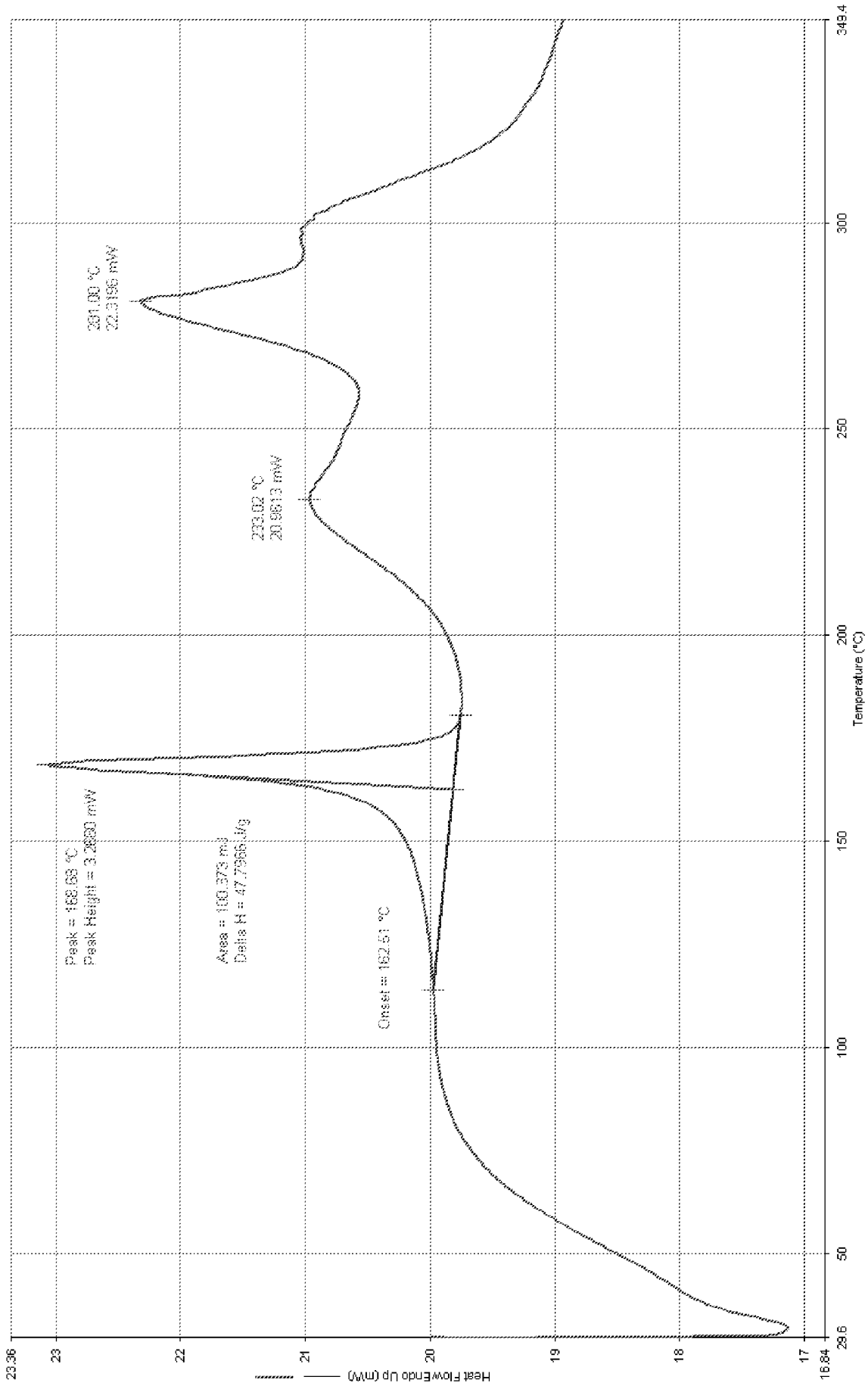

Peak = 168.68 °C
Peak Height = 3.2830 mW
Area = 160.373 mJ
Delta H = 47.7366 J/g
Onset = 162.51 °C
233.02 °C
20.9813 mW
281.00 °C
22.3195 mW
Heat FlowEndo Up (mW)
Temperature (°C)
23.36
23
22
21
20
19
18
17
16.84
29.6
50
100
150
200
250
300
349.4

Figure 11

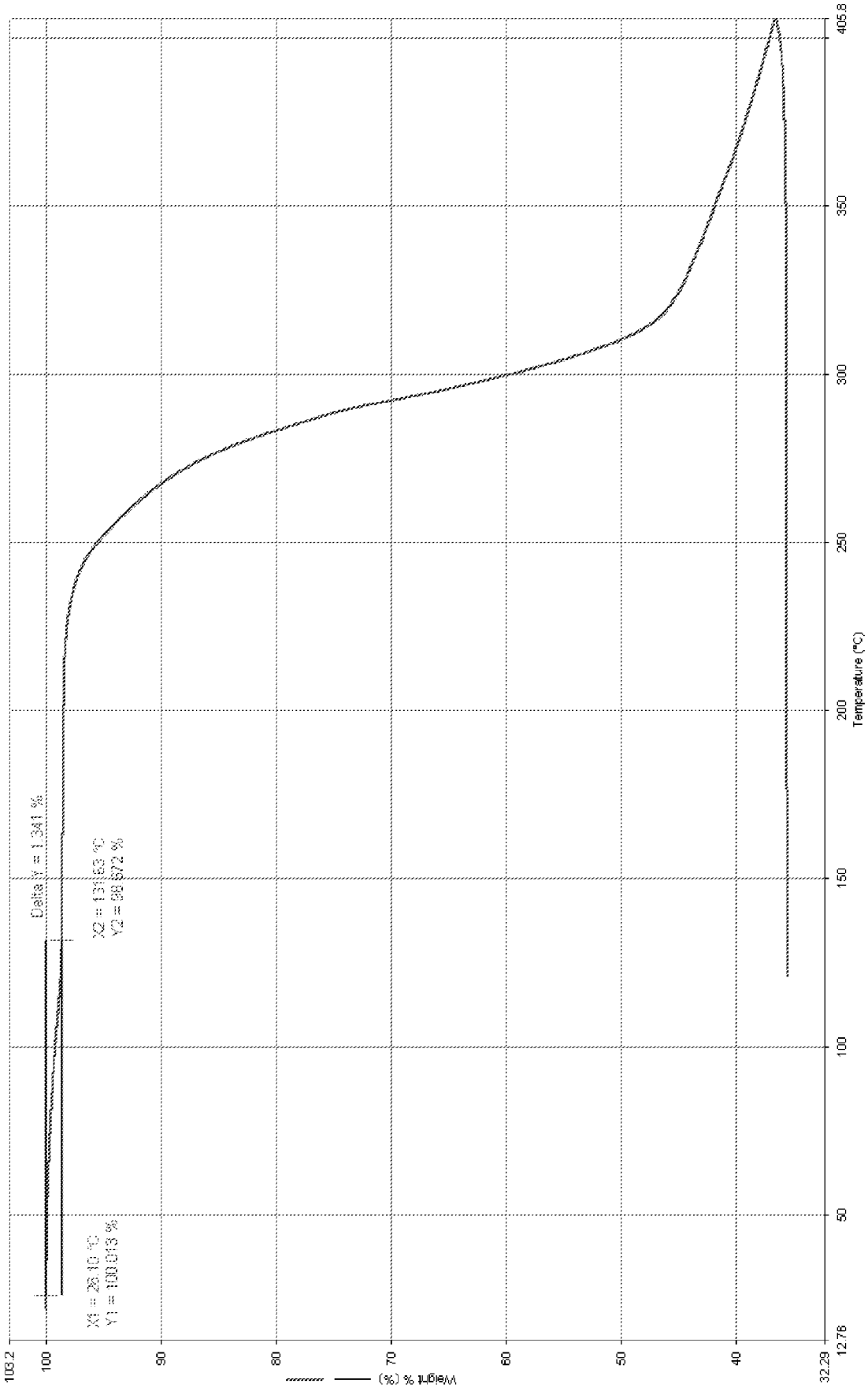

Delta y = 1.341 %
X2 = 131.53 °C
Y2 = 98.672 %
X1 = 26.10 °C
Y1 = 100.013 %
Weight % (%)
Temperature (°C)
103.2
100
90
80
70
60
50
40
32.29
12.76
50
100
150
200
250
300
350
405.8

Figure 12

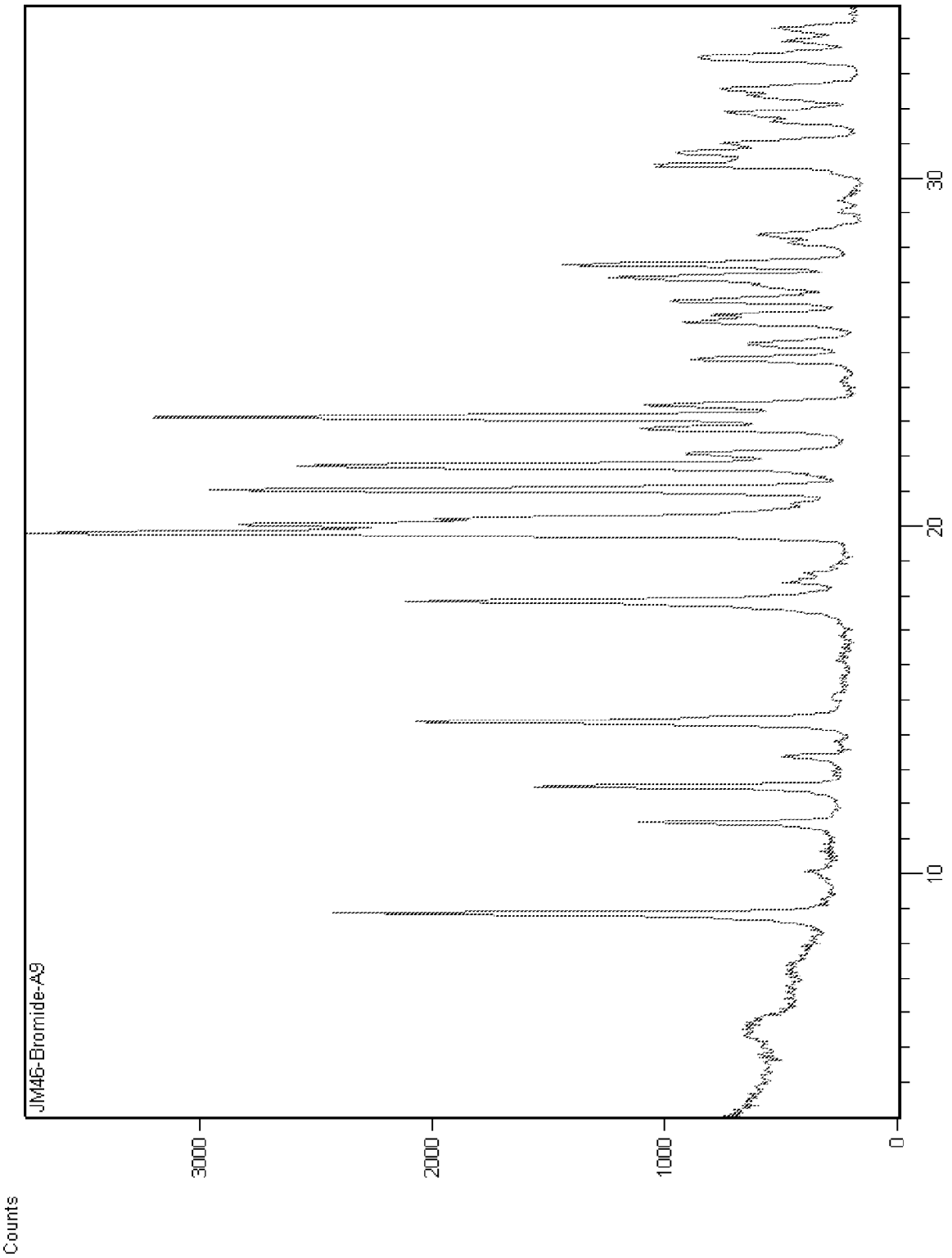

JM46-Bromide-A9
Counts
3000
2000
1000
0
10
20
30
Position [°2Theta] (Copper (Cu))

Figure 13

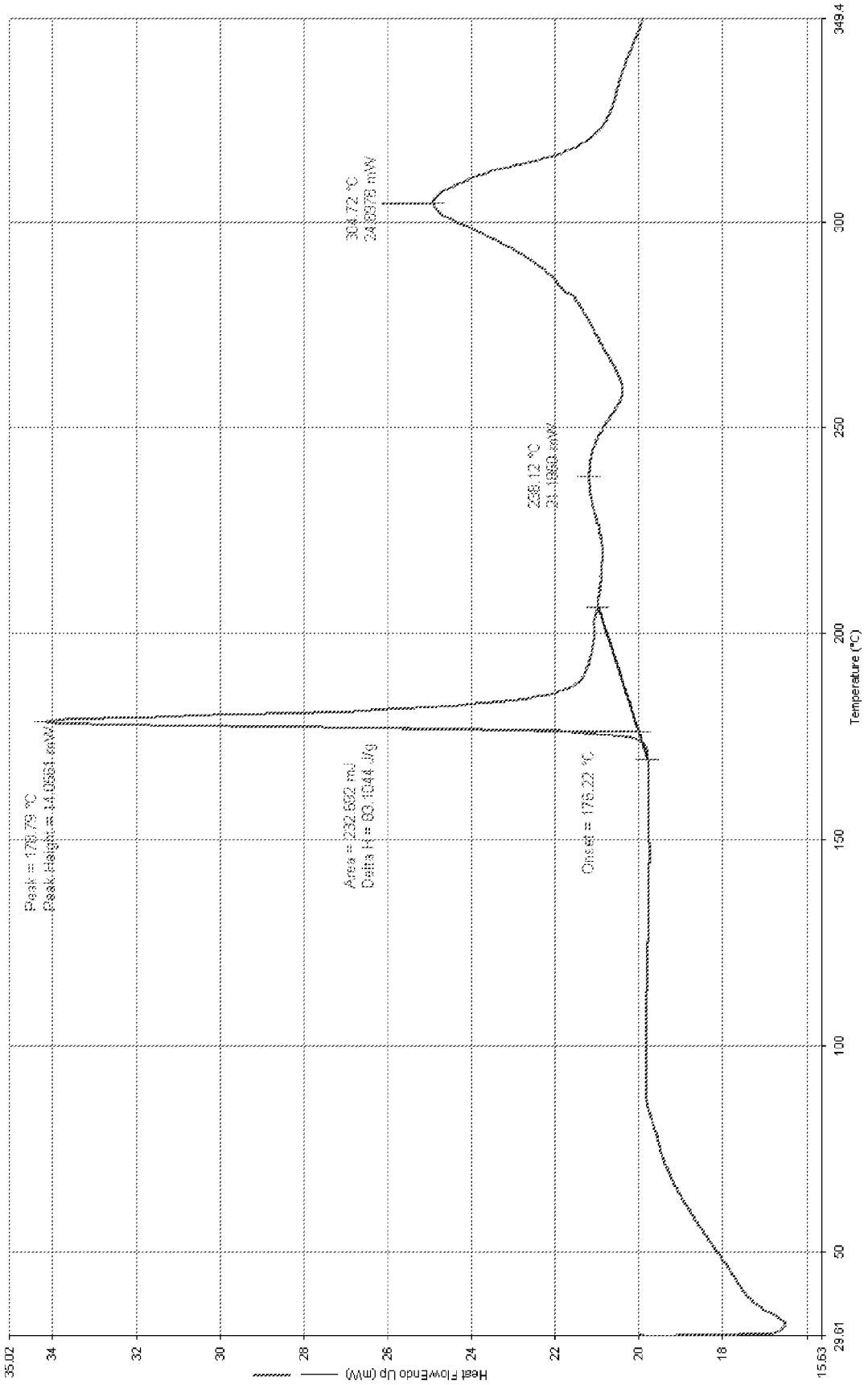

Peak = 178.79 °C
Peak Height = 34.05681 mW
Area = 232.592 mJ
Delta H = 93.1044 J/g
Onset = 176.22 °C
236.12 °C
304.72 °C
24.8978 mW
Heat FlowEndo Up (mW)
Temperature (°C)
35.02
34
32
30
28
26
24
22
20
18
15.63
29.61
50
100
150
200
250
300
349.4

Figure 14

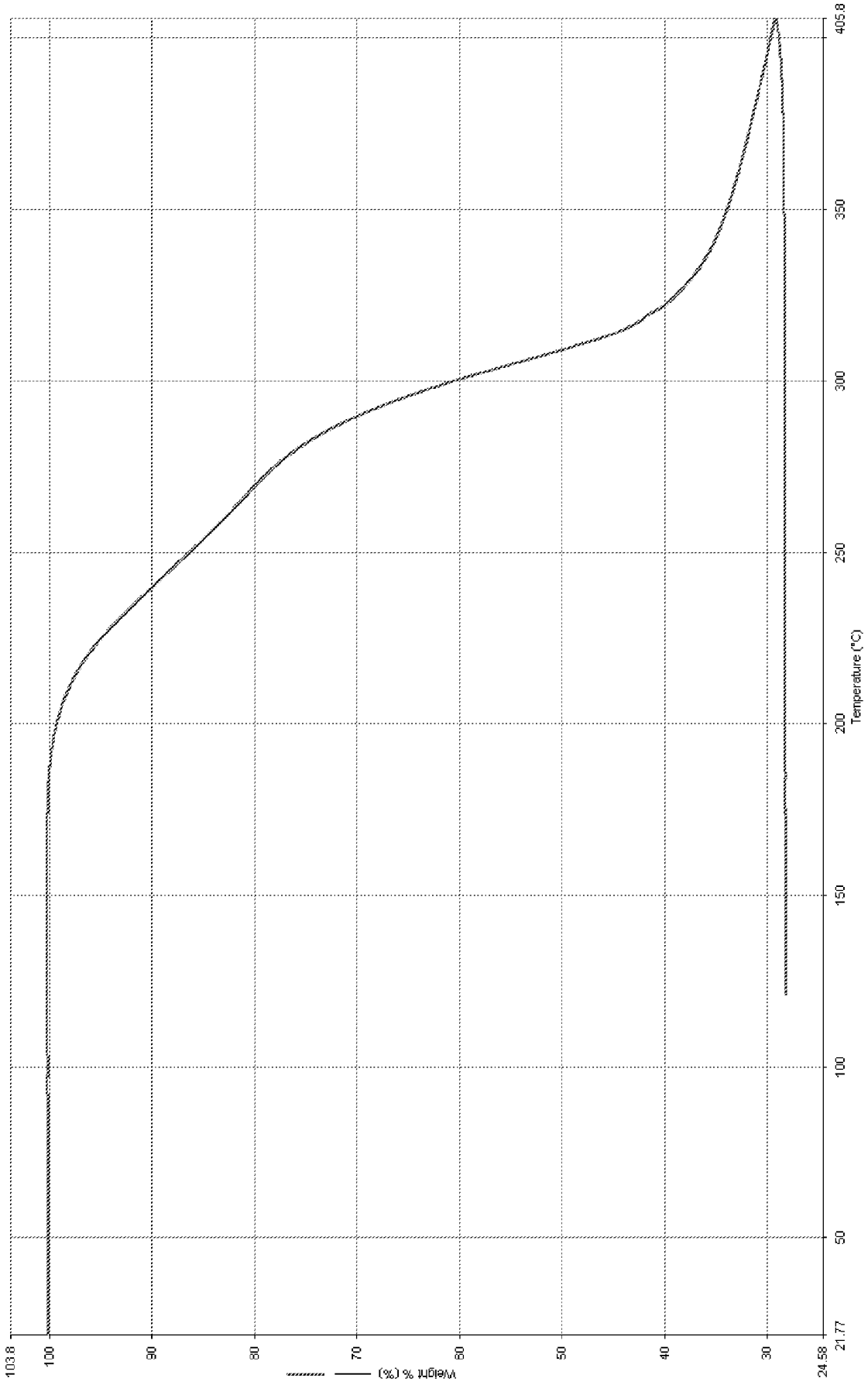

103.8
100
90
80
70
60
50
40
30
24.58
Weight % (%)
405.8
350
300
250
200
150
100
50
21.77
Temperature (°C)

Figure 15

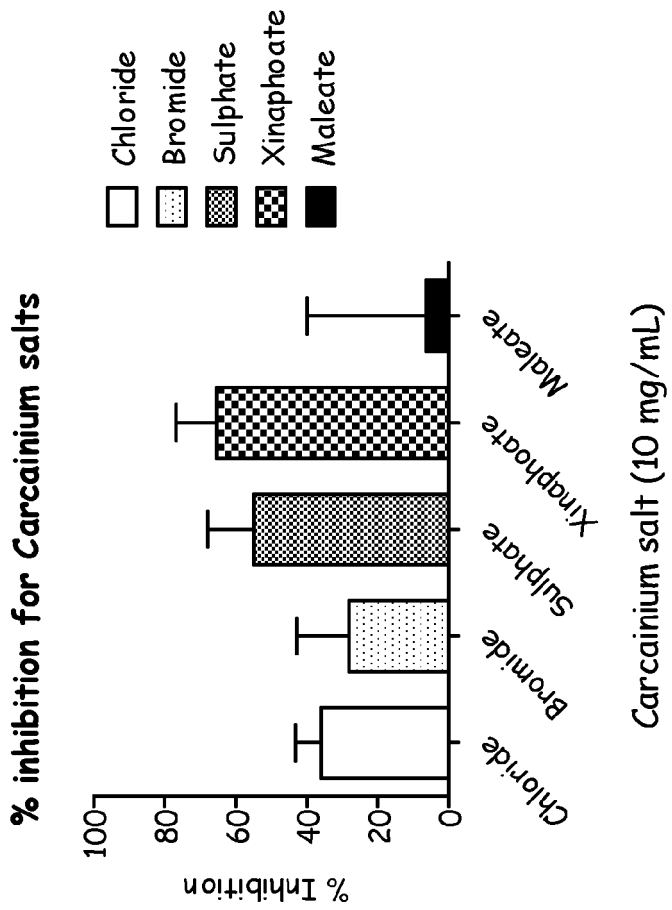

% inhibition for Carcainium salts
Chloride
Bromide
Sulphate
Xinaphoate
Maleate
% Inhibition
100
80
60
40
20
0
Chloride
Bromide
Sulphate
Xinaphoate
Maleate
Carcainium salt (10 mg/mL)

Figure 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/052325

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/72 A61K31/167 A61P11/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 362 197 B1 (PAGE CLIVE P [GB] ET AL) 26 March 2002 (2002-03-26) cited in the application column 1, line 7 - line 61 column 2, line 56 - line 67 column 3, line 1 - page 4, line 2 page 6, line 43 - line 54 column 6, line 65 - column 7, line 4 examples claims ----- -/--	1-18

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2013	26/11/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Epskamp, Stefan

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/052325

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J J ADCOCK ET AL: "RSD931, a novel anti-tussive agent acting on airway sensory nerves", BRITISH JOURNAL OF PHARMACOLOGY, vol. 138, no. 3, 1 February 2003 (2003-02-01), pages 407-416, XP055042457, ISSN: 0007-1188, DOI: 10.1038/sj.bjp.0705056 abstract page 408, left-hand column, last paragraph page 408, right-hand column, paragraph 5 - page 411, left-hand column, paragraph 2 page 411, left-hand column, paragraph 3 - page 414, right-hand column, paragraph 1 page 415, right-hand column, last paragraph -----	1,4,5, 14-18

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2013/052325

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6362197 B1	26-03-2002	AU 762127 B2	19-06-2003
		AU 4127299 A	30-12-1999
		BR 9911094 A	30-10-2001
		CA 2334481 A1	16-12-1999
		CN 1311673 A	05-09-2001
		EP 1087760 A1	04-04-2001
		JP 2002517438 A	18-06-2002
		US 6362197 B1	26-03-2002
		WO 9963985 A1	16-12-1999