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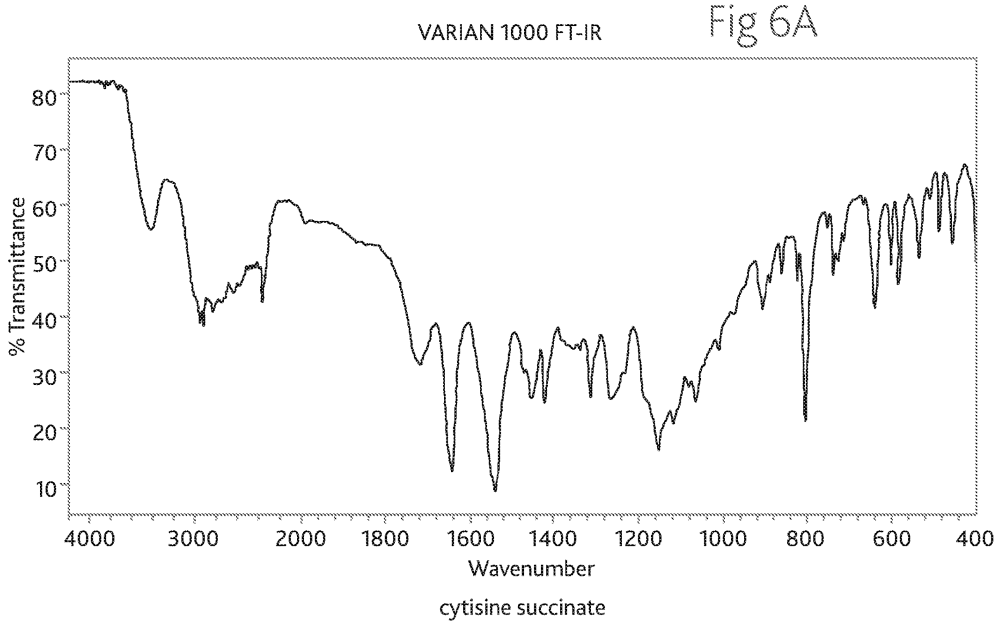
(56) Documents Cited:
WO 2015/025718 A1 **CN 104710422 A**
US 20160199315 A1 **US 20100048606 A1**

(58) Field of Search:
INT CL **A61K, A61P, C07D**
Other: **BIOSIS, EPODOC, MEDLINE, WPI, XPESP, XPSPRNG**

(54) Title of the Invention: **Salt**
Abstract Title: **Cytisine hydrogen succinate and the use thereof for the treatment of nicotine addiction, when mixed with a pharmaceutically acceptable excipient**

(57) Disclosed is a succinate salt of cytisine, cytisine hydrogen succinate, which is a nicotinic acetylcholine receptor agonist. It appears that cytosine hydrogen succinate has enhanced stability relative to cytisine free base, which is marketed as Tabex (RTM). The enhanced stability allows for both longer shelf-life and a wider range of, more conventional, excipients to be used. Also disclosed are solvates and hydrates of succinate salts of cytisine and mixtures comprising cytisine hydrogen succinate and pharmaceutically acceptable excipients; the resultant pharmaceutical composition may be formulated as a tablet or capsule. Preferably the excipient is lactose, corn starch or wheat starch; most preferably, the excipient is lactose. The pharmaceutical composition may be used in the treatment of nicotine addiction; most preferably providing a unit dose of cytisine succinate of 1 to 3 milligrams

Fig 6A



At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

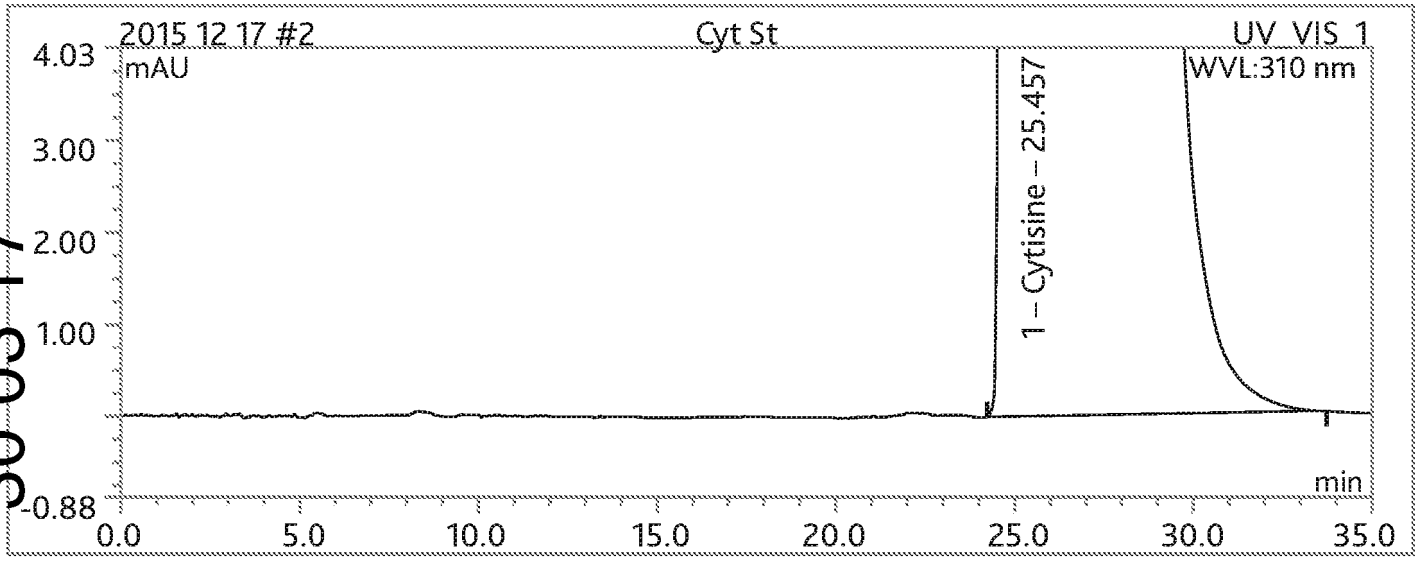
GB 2550241 A

Fig 1A

2 Cyt St

13.0mg Cytisine River 20121001C – 10 MPh

Sample Name:	**Cyt St**	*Injection Volume:*	**20.0**
Vial Number:	**RA2**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**310.0**
Control Program:	**Cytisine Salts**	*Bandwidth:*	**4**
Quantif. Method:	**Cytisine Salts**	*Dilution Factor:*	**1.0000**
Run Time (min):	**35.00**	*Sample Amount:*	**1.0000**



30 03 17

No.	Ret.Time min	Peak Name	RRT	Area mAU*min	Asymm	Plates(EP)	Res
1	25.46	Cytisine	1.00	1194.40380	2.00	1410	n.a.
Total:			1.000	1194.404	2.00	1410.000	

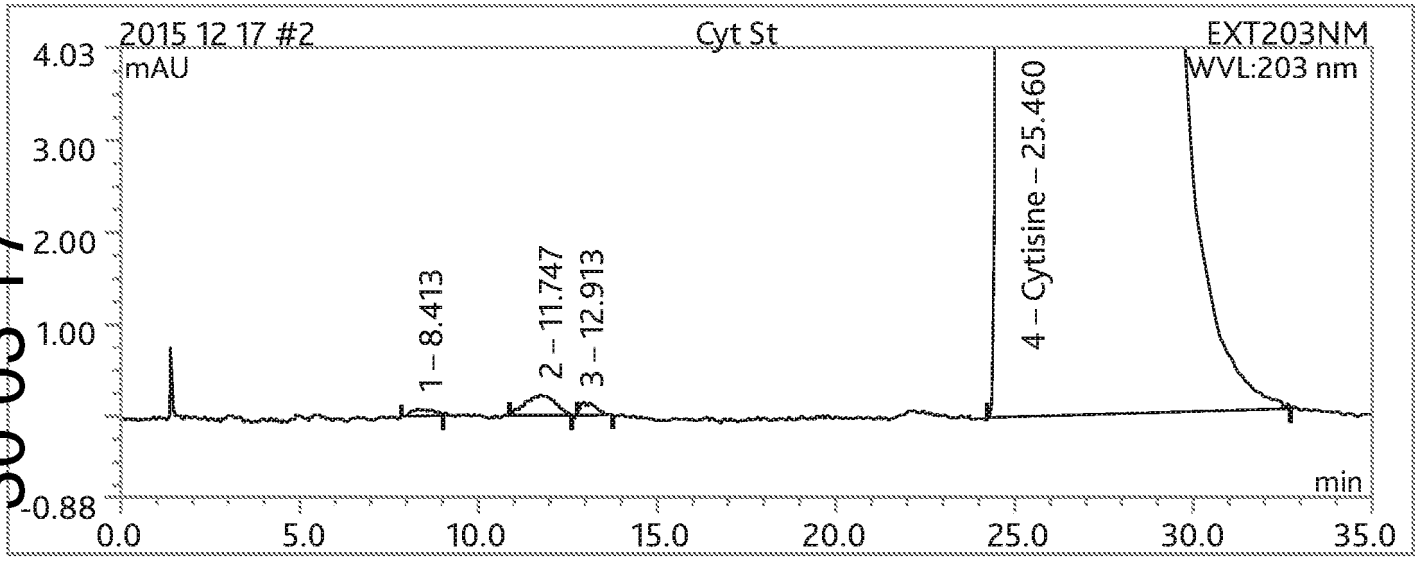
HPLC chromatogram of Cytisine (1mg/mL in Mobile Phase) at **310** nm

Fig 1B

2 Cyt St

13.0mg Cytisine River 20121001C – 10 MPh

Sample Name:	**Cyt St**	*Injection Volume:*	**20.0**
Vial Number:	**RA2**	*Channel:*	**EXT203NM**
Sample Type:	**unknown**	*Wavelength:*	**203**
Control Program:	**Cytisine Salts**	*Bandwidth:*	**0**
Quantif. Method:	**Cytisine Salts**	*Dilution Factor:*	**1.0000**
Run Time (min):	**35.00**	*Sample Amount:*	**1.0000**



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No.	Ret.Time min	Peak Name	RRT	Area mAU*min	Asymm	Plates(EP)	Res
1	8.41	n.a.	0.33	0.05599	0.98	949	2.41
2	11 .75	n.a.	0.46	0.22183	0.97	777	0.92
3	12.91	n.a.	0.51	0.07574	2.75	3573	6.54
4	25.46	Cytisine	1.00	1153.17886	2.09	1166	n.a.
Total:			2.299	1153.532	6.79	6465.000	

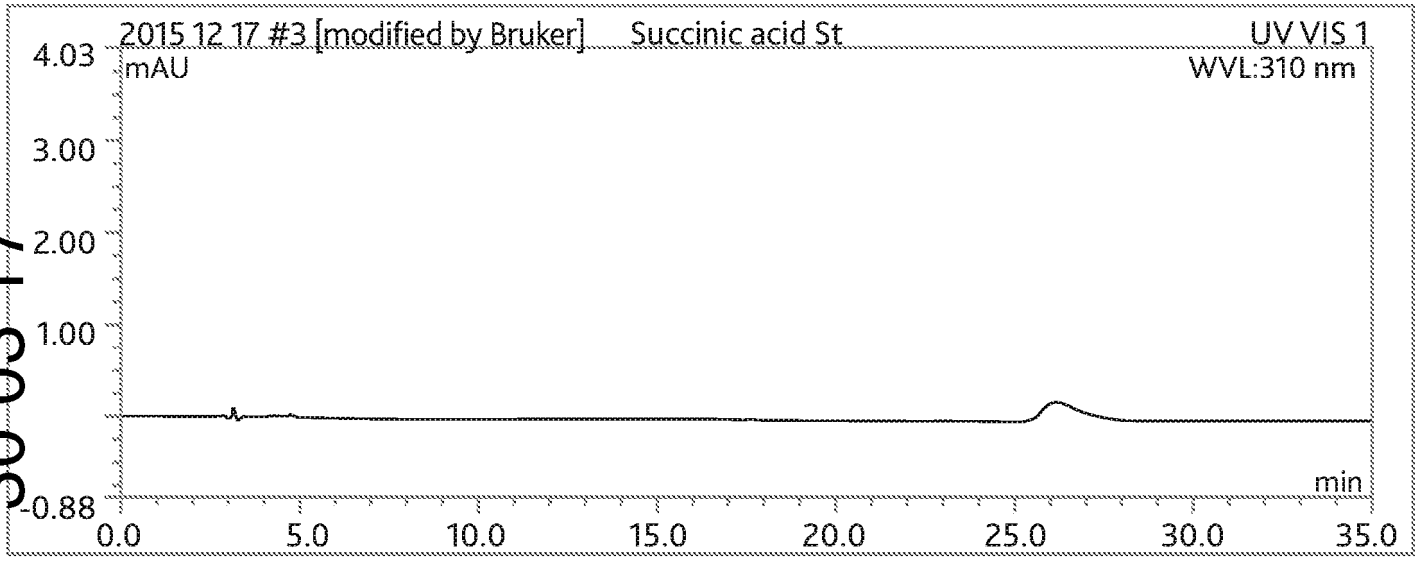
HPLC chromatogram of Cytisine (1mg/mL in Mobile Phase) at **203** nm

Fig 2A

3 Succinic acid St

11.8mg – 10 MPh

Sample Name:	**Succinic acid St**	*Injection Volume:*	**20.0**
Vial Number:	**RA3**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**310.0**
Control Program:	**Cytisine Salts**	*Bandwidth:*	**4**
Quantif. Method:	**Cytisine Salts**	*Dilution Factor:*	**1.0000**
Run Time (min):	**35.00**	*Sample Amount:*	**1.0000**



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No.	Ret.Time min	Peak Name	RRT	Area mAU*min	Asymm	Plates(EP)	Res
Total:			0.000	0.000	0.00	0.000	

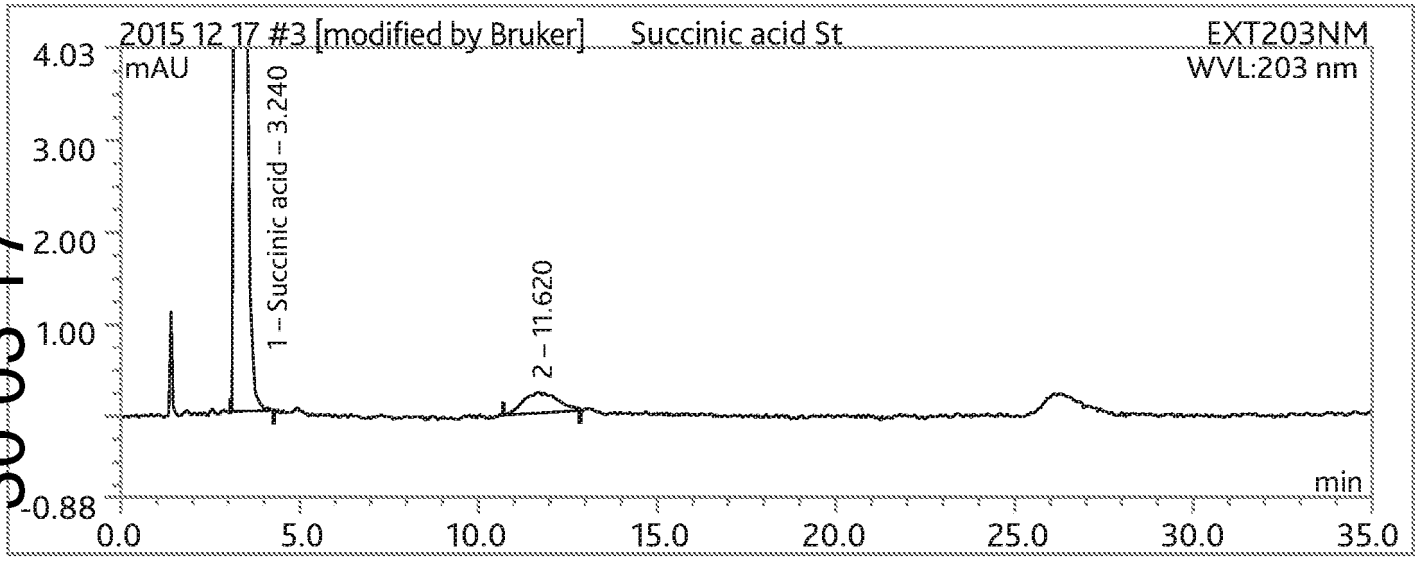
HPLC chromatogram of Succinic acid (1mg/mL in Mobile Phase) at **310** nm

Fig 2B

3 Succinic acid St

11.8mg – 10 MPh

Sample Name:	**Succinic acid St**	*Injection Volume:*	**20.0**
Vial Number:	**RA3**	*Channel:*	**EXT203NM**
Sample Type:	**unknown**	*Wavelength:*	**203**
Control Program:	**Cytisine Salts**	*Bandwidth:*	**0**
Quantif. Method:	**Cytisine Salts**	*Dilution Factor:*	**1.0000**
Run Time (min):	**35.00**	*Sample Amount:*	**1.0000**



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No.	Ret.Time min	Peak Name	RRT	Area mAU*min	Asymm	Plates(EP)	Res
1	3.24	Succinic acid	n.a.	18.40723	1.70	2710	8.10
2	11.62	n.a.	n.a.	0.24803	1.23	649	n.a.
Total:			0.000	18.655	2.92	3359.000	

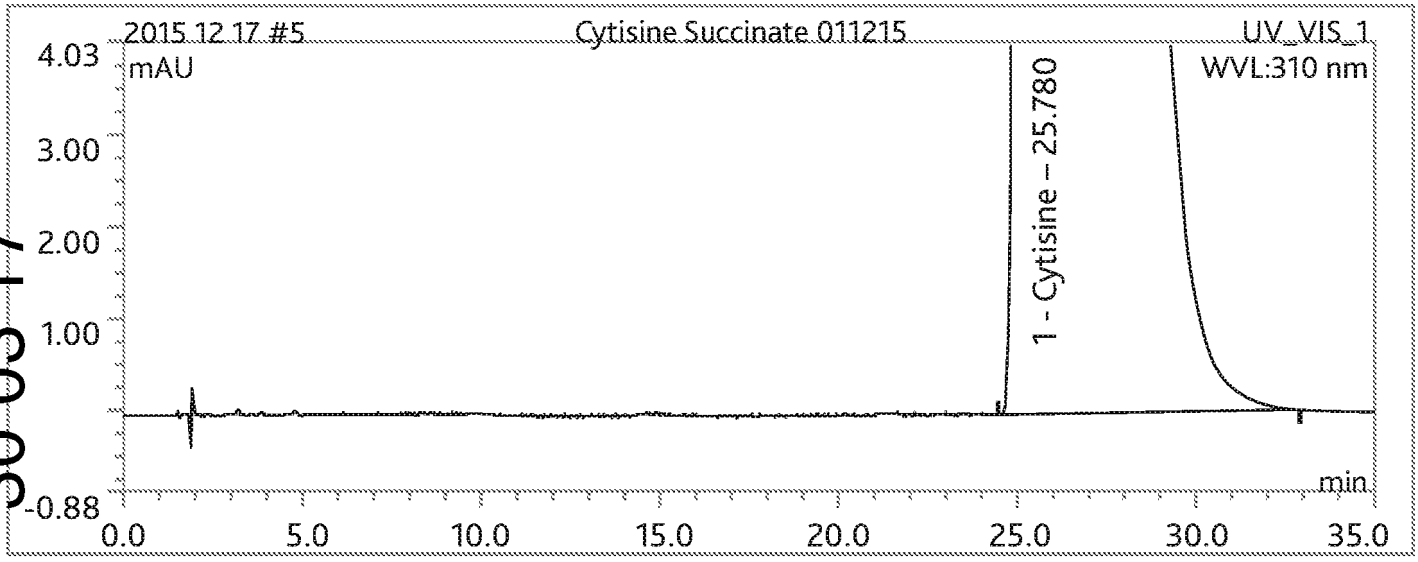
HPLC chromatogram of Succinic acid (1mg/mL in Mobile Phase) at **203** nm

Fig 3A

5 Cytisine Succinate

11.26mg-10MPh

Sample Name:	**Cytisine Succinate**	*Injection Volume:*	**20.0**
Vial Number:	**RA5**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**310.0**
Control Program:	**Cytisine Salts**	*Bandwidth:*	**4**
Quantif. Method:	**Cytisine Salts**	*Dilution Factor:*	**1.0000**
Run Time (min):	**35.00**	*Sample Amount:*	**1.0000**



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No.	Ret.Time min	Peak Name	RRT	Area mAU*min	Asymm	Plates(EP)	Res
1	25.78	Cytisine	1.00	574.58035	1.97	1954	n.a.
Total:			1.000	574.580	1.97	1954.000	

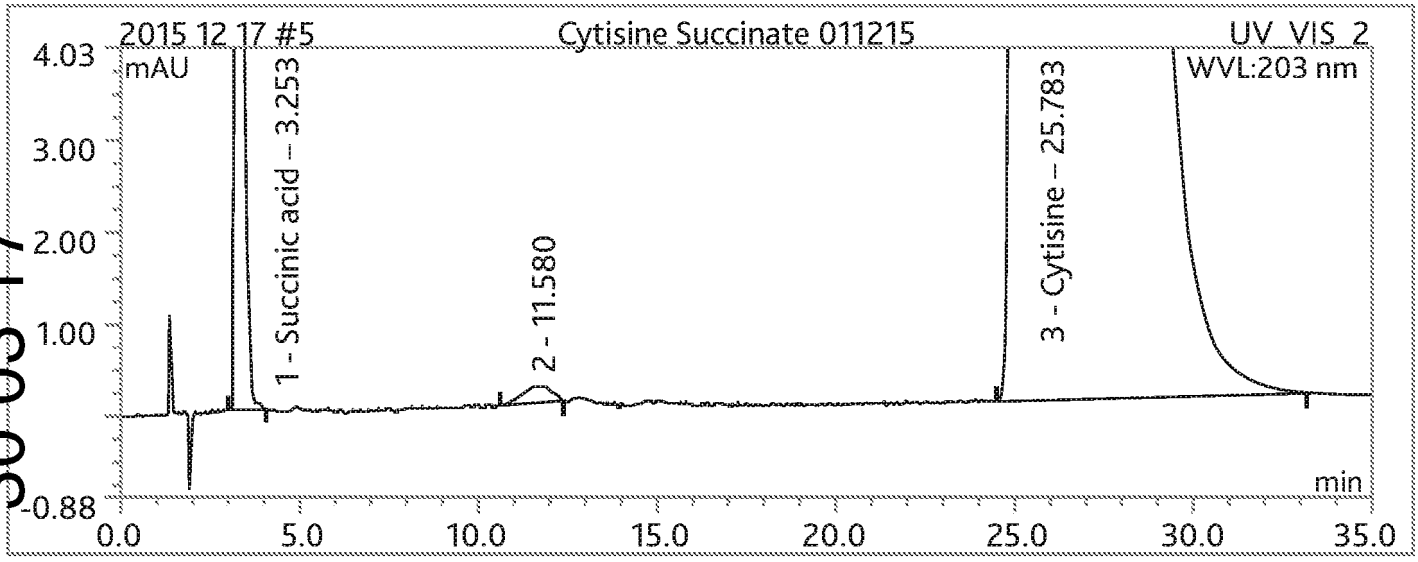
HPLC chromatogram of Cytisine Succinate (1mg/mL in Mobile Phase) at **310** nm

Fig 3B

5 Cytisine Succinate

11.26mg-10MPh

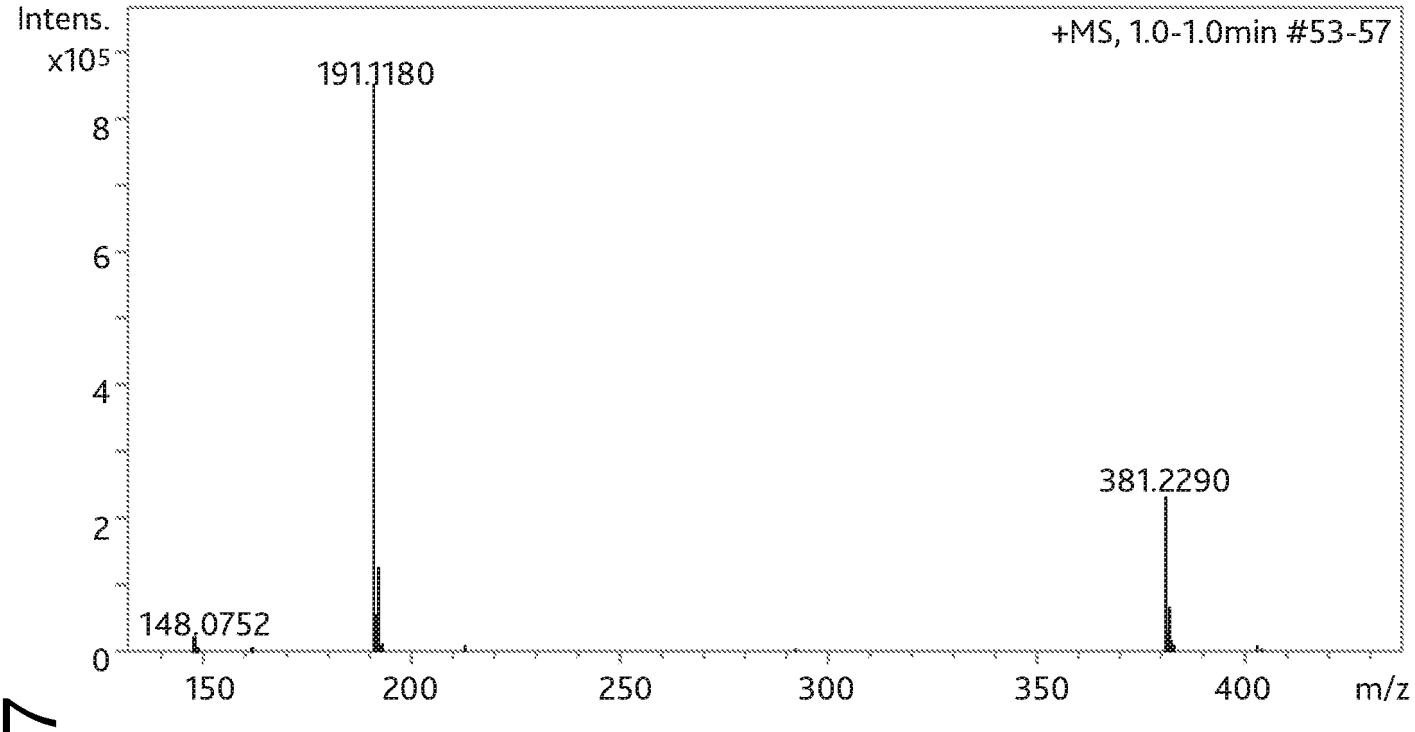
Sample Name:	**Cytisine Succinate**	*Injection Volume:*	**20.0**
Vial Number:	**RA5**	*Channel:*	**UV_VIS_2**
Sample Type:	**unknown**	*Wavelength:*	**203.0**
Control Program:	**Cytisine Salts**	*Bandwidth:*	**4**
Quantif. Method:	**Cytisine Salts**	*Dilution Factor:*	**1.0000**
Run Time (min):	**35.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	RRT	Area mAU*min	Asymm	Plates(EP)	Res
1	3.25	Succinic acid	0.13	7.40247	1.65	2963	9.28
2	11 .58	n.a.	0.45	0.16655	0.97	882	7.02
3	25.78	Cytisine	1.00	596.35369	2.03	1709	n.a.
Total:			1.575	603.923	4.65	5554.000	

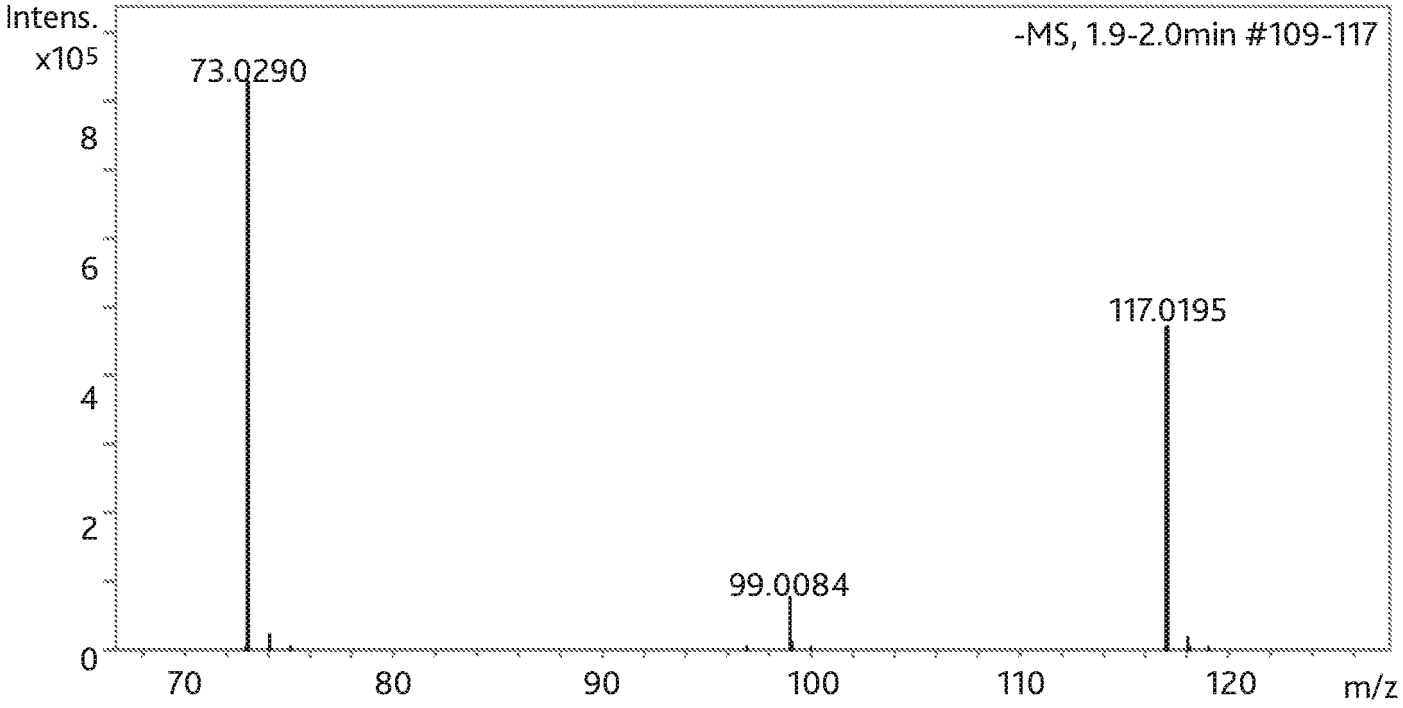
HPLC chromatogram of Cytisine Succinate (1mg/mL in Mobile Phase) at **203** nm

Fig 4A



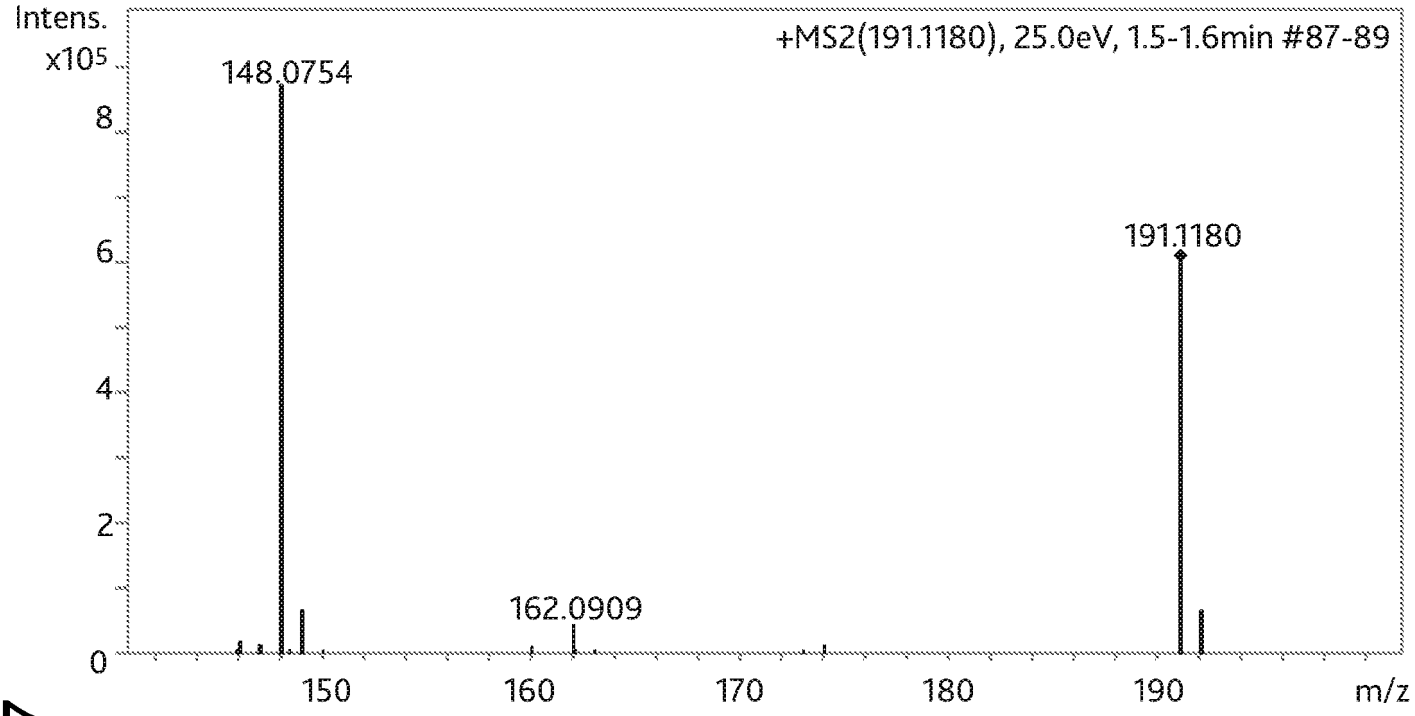
HR ESI+ MS Spectrum of Cytisine Succinate (191. 1180 Cyt+H+; 381.2290 2Cyt+H+)

Fig 4B



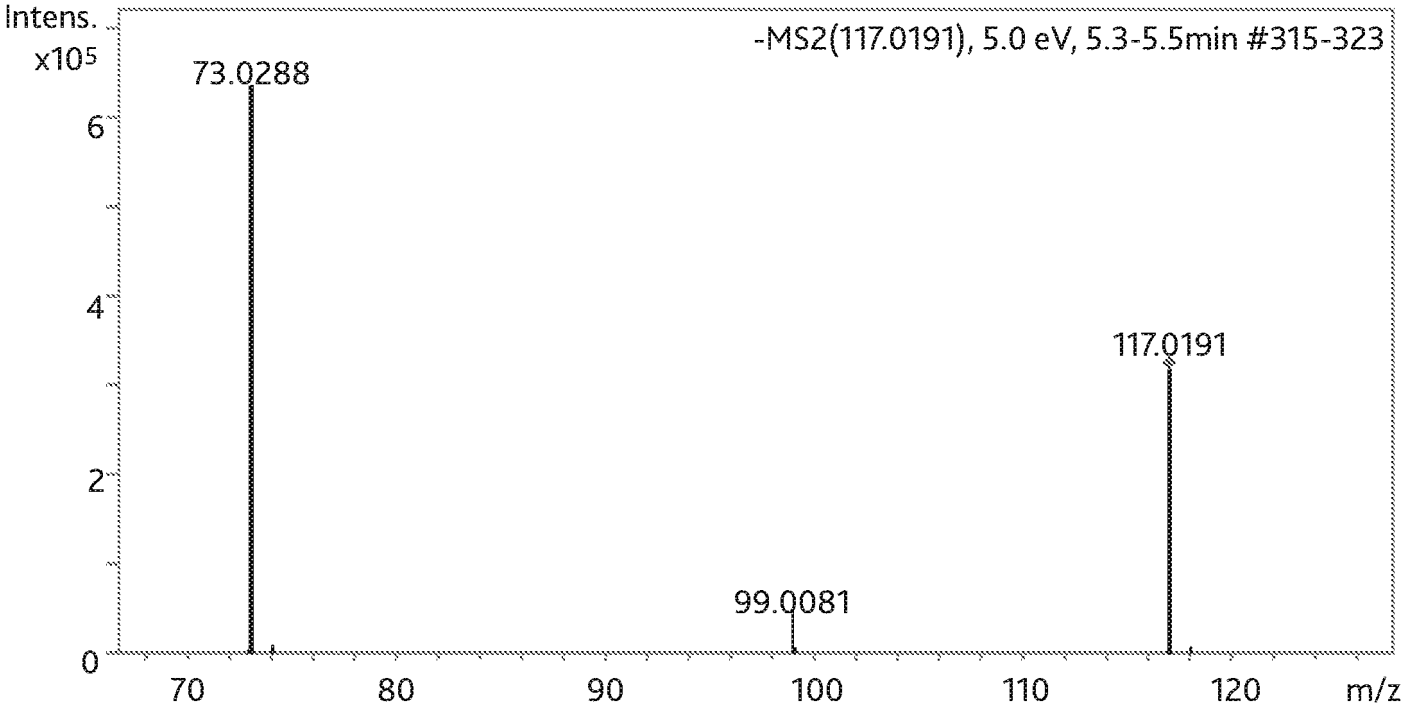
HR ESI- MS Spectrum of Cytisine Succinate (117.0195 Succ-H+; 99.0084 Succ-H2O-H+; 73.0290 Succ-CO2-H+)

Fig 5A



HR ESI+ MS/MS Spectrum of 191.1180 (**191.1180** Cyt+H$^+$; 148.0754 Cyt-CH_2NHCH_2+H$^+$)

Fig 5B



HR ESI- MS/MS Spectrum of **117.0191** (117.0195 Succ-H$^+$; 99.0084 Succ-H_2O-H$^+$; 73.0290 Succ-CO_2-H$^+$)

Fig 6A

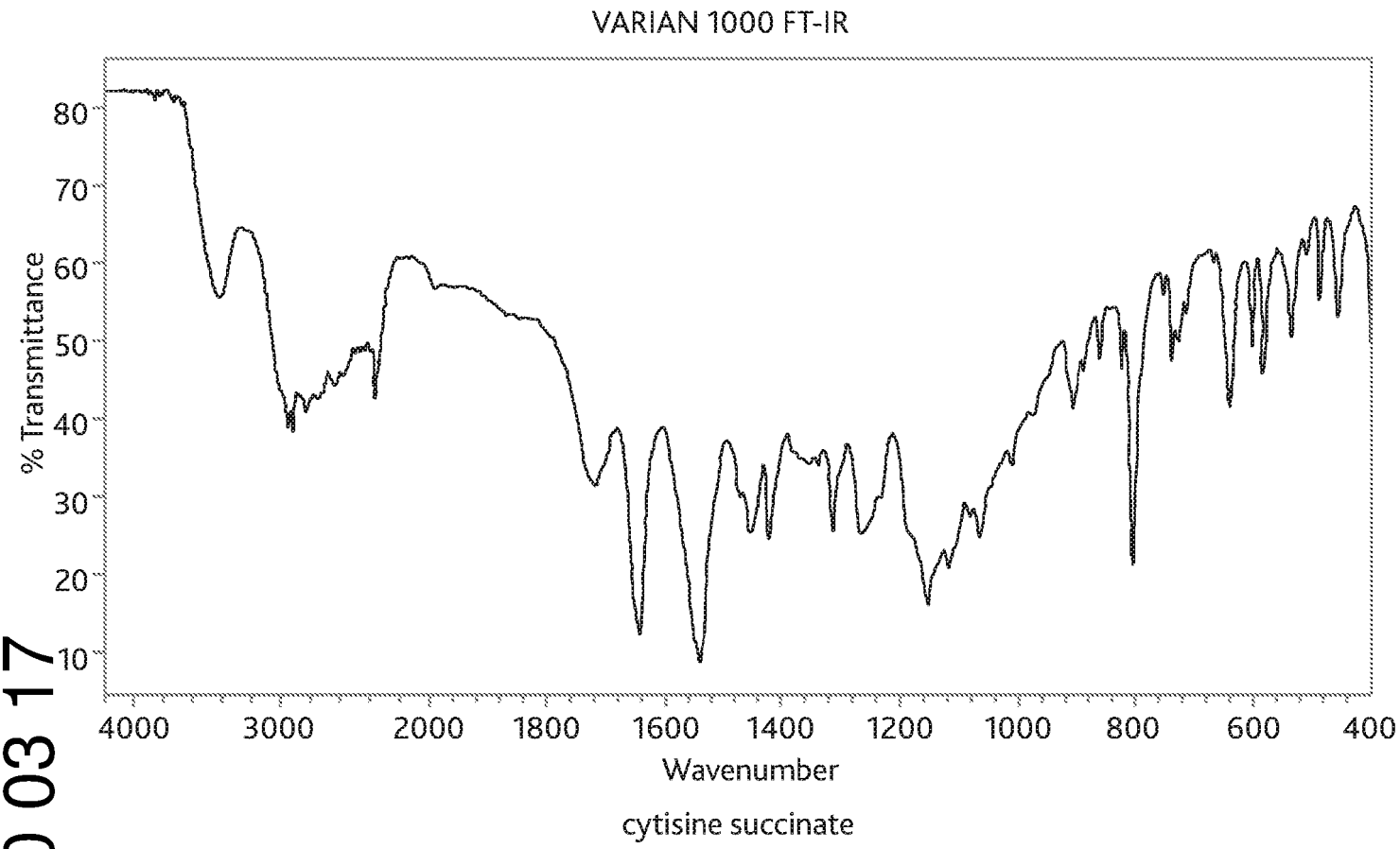


Fig 6B

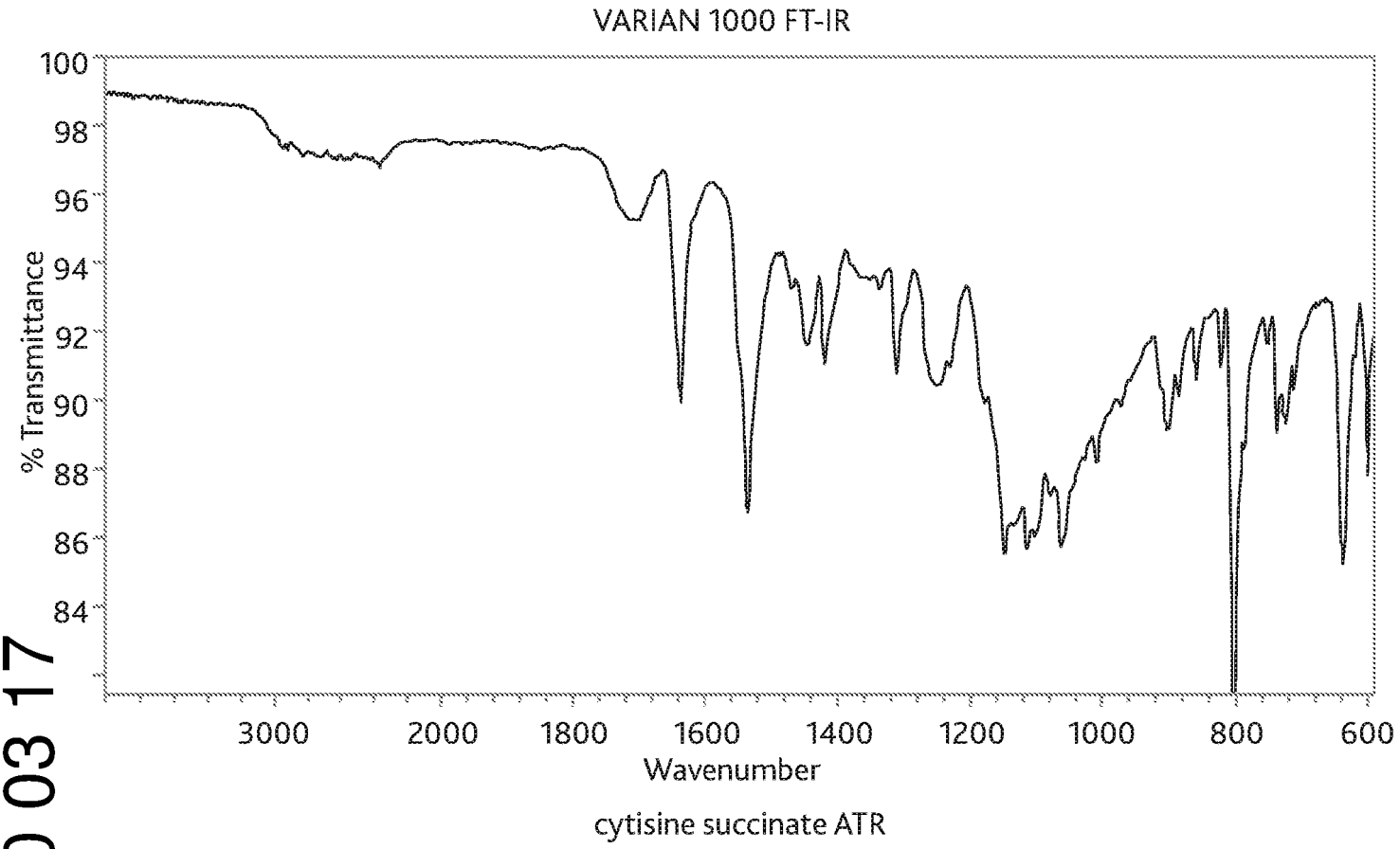
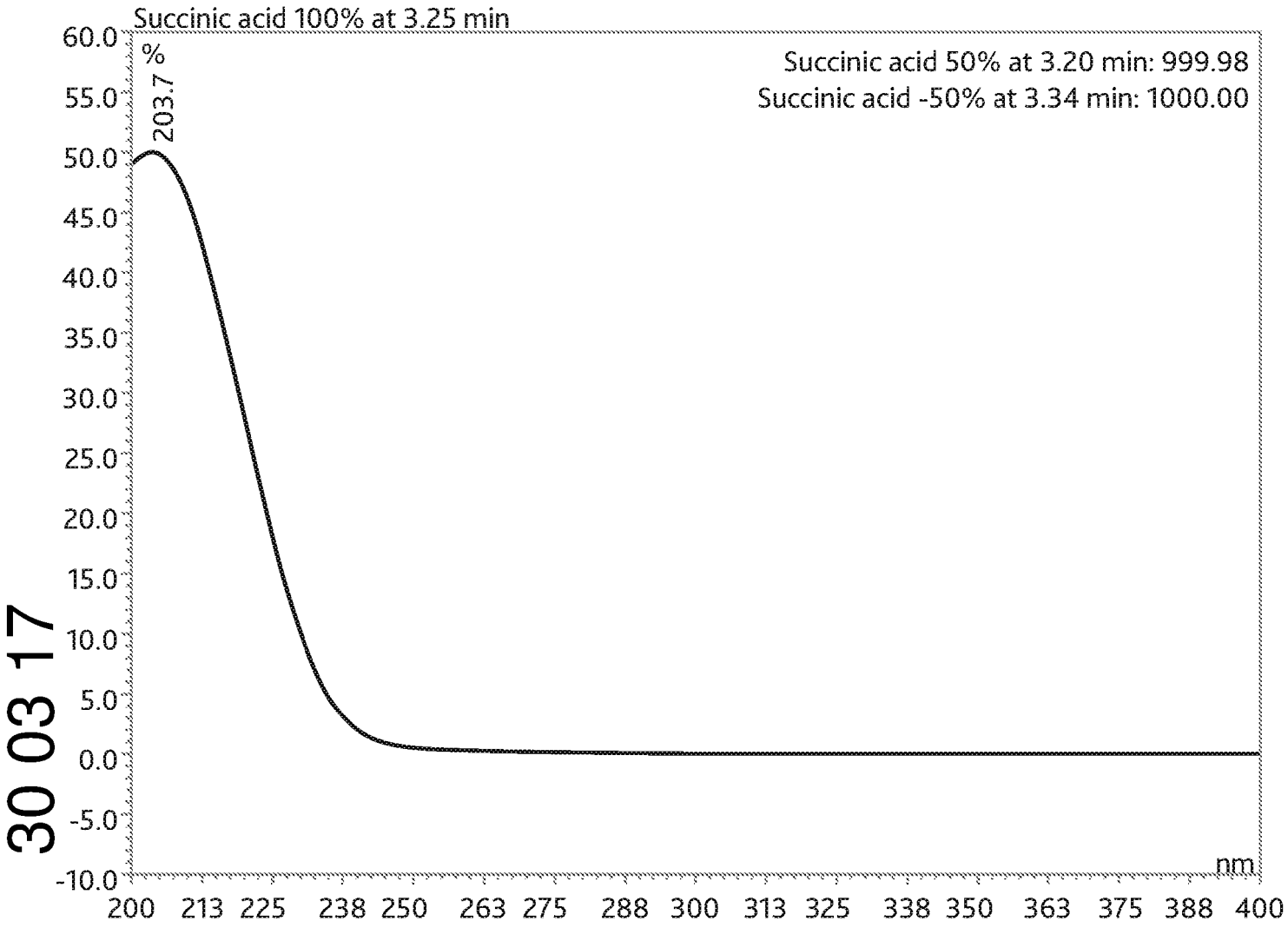
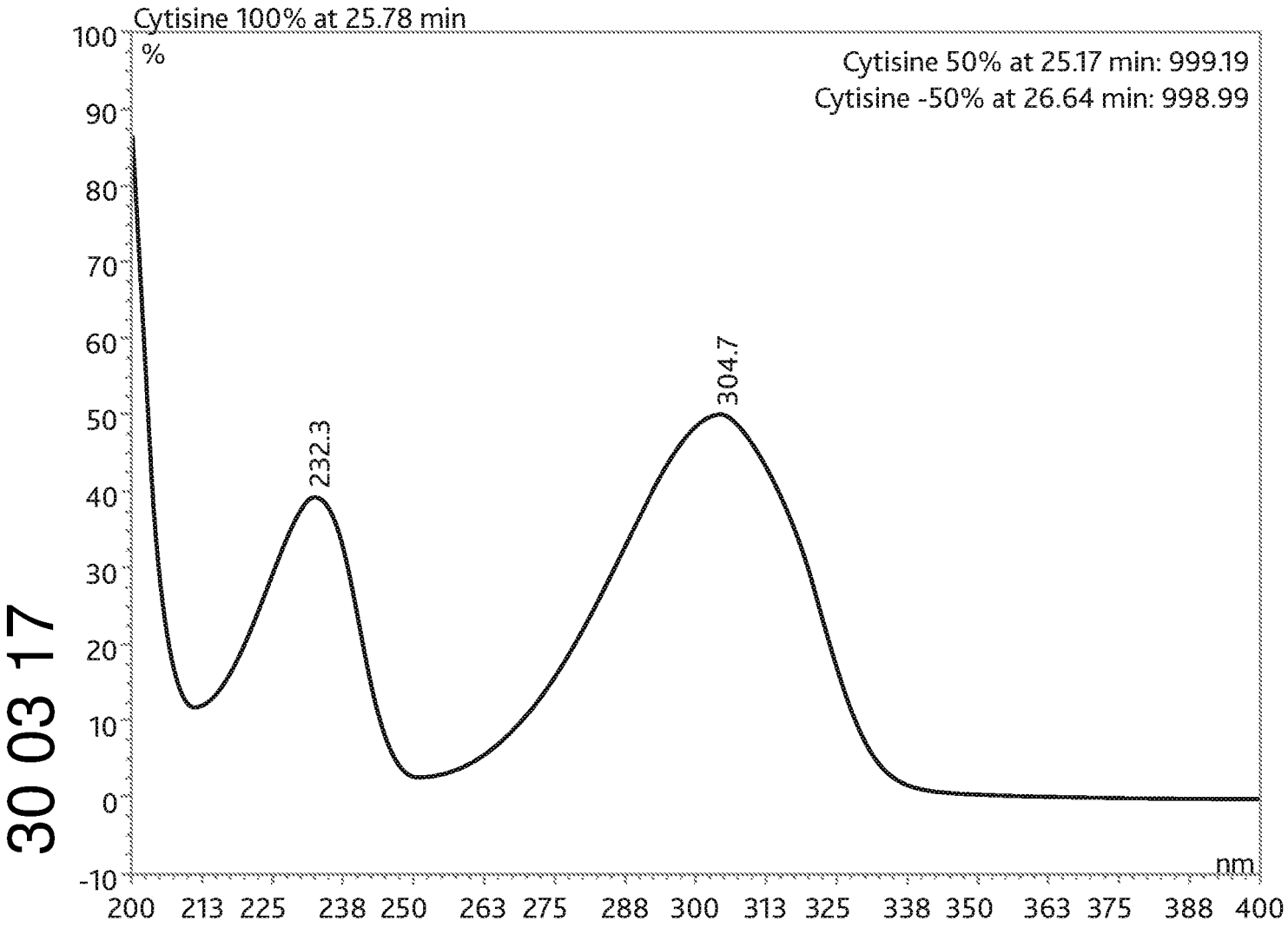


Fig 7A



Peak purity of the signal with RT 3,25 min (Succinic acid) and the corresponding UV-VIS spectrum recorded with DAD

Fig 7B



Peak purity of the signal with RT 25,78 min (Cytisine) and the corresponding UV-VIS spectrum recorded with DAD

Salt

The present invention relates to a novel salt of cytisine. The invention also relates to pharmaceutical compositions comprising a novel cytisine salt.

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

A pharmaceutical smoking cessation product containing cytisine 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 cytisine 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 shelf-life is exceeded. Additionally, where the product is to be shipped to territories with higher temperature / humidity climates (e.g. ICH climactic zones III and IV), then additional packaging to protect the product and maintain shelf-life may be required.

A number of formulation approaches for cytisine-containing products are proposed in the prior art. For example, EP1586320 discloses a solid dosage formulation comprising cytisine free base. While it is stated in that document that the disclosed formulation provides improved stability, there is no suggestion that this could be achieved by the use of salts of cytisine. Indeed, no salts of cytisine are disclosed in that document.

WO2014/076680 discloses a cytisine-containing formulation which again is stated as improving the stability of cytisine. As with EP1586320, there is no suggestion of using a salt of cytisine to improve stability, nor are any salts mentioned. In WO2014/076680 the issue of incompatibility between the cytisine active ingredient and lactose is raised. Specifically, it is stated that lactose may destabilise tablets comprising cytisine 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.

Accordingly, there is a need in the art for a form of cytisine which inherently is more compatible with conventional excipients such as lactose.

The present inventors have surprisingly and unexpectedly identified a novel salt of cytisine which displays improved excipient compatibility and can be formulated with lactose as an excipient.

Thus, according to a first aspect of the present invention, there is provided a succinate salt of cytisine. In embodiments of the present invention, the salt may be present in the form of a solvate or a hydrate. The salt is preferably cytisine hydrogen succinate.

As mentioned above, the improved compatibility of the salt permits the preparation of stable pharmaceutical compositions. Thus, according to a second aspect of the present invention, there is provided a pharmaceutical composition comprising the succinate salt of cytisine and a pharmaceutically acceptable carrier.

In an embodiment the pharmaceutically acceptable carrier is lactose. The lactose may be lactose monohydrate or anhydrous lactose.

The compositions disclosed herein may be suitable for administration by any route known in the art. Pharmaceutical formulations 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.

In terms of excipients that may be employed in the compositions of the present invention, these include fillers, disintegrants, preserving agents, lubricants (e.g. magnesium stearate) and / or wetting agents. Examples of fillers that may be used include lactose (either anhydrous or monohydrate), cellulose, starch (e.g. corn and / or wheat starch), calcium phosphates, mannitol and others known in the art.

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

The pharmaceutical composition may be coated according to any method known in the art, for example using collidone or shellac, gum arabic, talc, titanium dioxide or sugar.

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

In embodiments of the invention in which the pharmaceutical composition is provided in the form of capsules, these may be prepared by any suitable method. For example, such capsules may be prepared by mixing the salts with inert carriers such as lactose or sorbitol and packing them into gelatine capsules.

In embodiments of the invention, the pharmaceutical composition will be provided as a unit dosage form (e.g. a tablet, capsule). The amount of cytisine succinate salt in the composition may range from about 0.5mg or about 1.0mg to 2.0mg, 3.0mg, 5.0mg or about 10mg. 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%.

Figures

Figure 1 shows HPLC chromatograms of cytisine at (a) 310nm and (b) 203nm.

Figure 2 shows HPLC chromatograms of succinic acid at (a) 310nm and (b) 203nm.

Figure 3 shows HPLC chromatograms of cytisine succinate at (a) 310nm and (b) 203nm.

Figure 4 shows the HR MS spectra of cytisine succinate: (a) ESI+ and (b) ESI-

Figure 5 shows the MS-MS spectra of cytisine succinate: (a) ESI+ and (b) ESI-

Figure 6 shows the IR spectra of cytisine succinate: (a) FTIR and (b) FTIR (ATR)

Figure 7 shows the peak purity determinations using a DAD detector for cytisine hydrogensuccinate (a) succinic acid and (b) cytisine).

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

Example 1: Preparation of cytisine succinate salt

An aqueous mixture of cytisine and succinic acid was prepared. HPLC chromatograms for these starting materials are provided as Figures 1 and 2. Acetone was added, and cytisine succinate salt was isolated from the mixture at a yield of about 70%.

The starting materials (cytisine and succinic acid) and the obtained salt were characterised using a number of analytical methods, see Figures 1 to 7. The results are

presented in Table 1 below. Chromatographic purity of the salt was determined using HPLC and was found to be greater than 99.9% (Figure 3). As can be seen, the salt of the present invention can be easily produced at very high levels of purity using conventional salification processes.

The obtained salt was also subjected to high resolution mass spectrum analysis (Figure 4), tandem mass spectroscopy (Figure 5), IR spectroscopy (Figure 6) and UV / VIS analysis (Figure 7) was performed.

Table 1 Characterisation tests

№	Test items	Cytisine Succinate
1	Appearance	White crystals
2	UV/VIS (HPLC DAD)	
	UV/VIS Cytisine	Max. 232.3 nm; 304.7 nm
	UV/VIS Acid Anion	Max. 203.7 nm
3	IR	2932 cm^{-1}; 2363 cm^{-1}; 1719 cm^{-1}; 1645 cm^{-1}; 1545 cm^{-1}; 1267 cm^{-1}; 1161 cm^{-1}; 805 cm^{-1}; 642 cm^{-1}
4	HR MS (Q-TOF)	
	ESI+	191.1180 Cyt+H^+ 381.2290 2Cyt+H^+
	ESI-	117.0195 Succ-H^+ 99.0084 Succ-H_2O-H^+ 73.0290 Succ-CO_2-H^+
5	Chromatographic purity (HPLC DAD)	310 nm > 99.9%
		203 nm > 99.9%

Example 2: Stability of cytisine succinate salt

Samples of cytisine succinate salt obtained from Example 1 were formulated in standard formulations to investigate their stability, and the compatibility of the API with the excipients used. Preliminary analysis of these formulations indicates that the formulations comprising succinate salt are significantly more stable than those comprising cytisine free base.

Example 3 – Salt Screen

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

Acid	Solvent mix	Molarity
Acetate	EtOH	1
Ascorbate	9:1 EtOH:water	0.5
Benzoate	EtOH	1
Succinate	EtOH	0.5

Stock solutions of cytisine API (non-synthetic) were also prepared in CH_3CN and 2-Me-THF. 2.4g of cytisine was dissolved in 24ml of CH_3CN heated to a temperature of 40°C. 2.4g of cytisine was dissolved in 60ml of 2-Me-THF heated to a temperature of 60°C.

10ml capacity tubes heated to 40°C were charged with 2ml of the cytisine / CH_3CN stock solution or 5ml of the cytisine 2-Me-THF 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, manipulations were carried out, 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 were then analysed, and the following table summarises the outcome of this screen and the properties of the obtained products;

API Stock Solution	Salt-type	Assessment
CH_3CN	Acetate	Base precipitation, unstable salt
2-MeTHF	Acetate	Base precipitation, unstable salt
CH_3CN	Ascorbate	Base precipitation, unstable salt

2-MeTHF	Ascorbate	Base precipitation, unstable salt
CH_3CN	Benzoate	Failed to yield solid
2-MeTHF	Benzoate	Failed to yield solid
CH_3CN	Succinate	Excellent salt profile, mono succinate, reasonably high melt
2-MeTHF	Succinate	Equivalent succinate to CH_3CN example with phase impurity/thermally induced modulation, possible polymorphism

As can be seen, suitable salts could not be formed with the common acid salt formers acetic acid, ascorbic acid or benzoic acid. However, the succinate salt was readily formed, exhibiting advantageous properties.

Example 4 – Lactose Incompatibility Testing

Cytisine, 0.9541g, was dissolved in water, 1ml, and afforded a yellow solution. Succinic acid, 0.5919g, 1equiv, was charged as a solid to the cytisine solution and dissolved slowly with agitation. Acetone, 10ml, was charged and afforded a partitioned mixture of cytisine/succinic acid/water solution, lower, and acetone, upper. Trituration of a portion of cytisine/succinic acid/water solution with acetone, 10ml, converted the viscous mixture to a white solid which settled. The white suspension was charged to the remainder of the cytisine/succinic acid/water solution/acetone mixture with a rinse of acetone, 10ml, and agitation continued. This converted the viscous cytisine/succinic acid/water mixture to a white suspension which settled when agitation was stopped. The solid was isolated by filtration and dried in vacuo at 50°C for ca 16 hours. The recovered solid was confirmed as being cytisine succinate by ^1H NMR analysis.

Recovery: 1.5463g, 80.76% based upon a salt stoichiometry of cytisine to succinic acid of 1:1

The stability of cytisine / lactose and cytisine succinate / lactose binary mixtures was assessed at 40°C and 75% relative humidity (RH) in vials with loosened lids at 9 days.

The sample mixtures and storage conditions used are detailed in Table 2 and Table 3.

Table 2 Cytisine /lactose mixtures and storage conditions

No.	Cytisine, mg	Lactose, mg	Storage condition
1	100.6	99.8	40°C and 75% RH

Table 3 Cytisine succinate/lactose mixtures and storage conditions

No.	Cytisine succinate, mg	Lactose, mg	Storage condition
2	98.4	101.8	40°C and 75% RH

The chemical purity of a cytisine/lactose mixture was 99.83 area% and a cytisine succinate/lactose mixture was 99.68 area% at the start of the of the stability study.

Upon completion of the stability testing period, the following results were observed:

Table 4 Characteristics of cytisine, cytisine succinate, cytisine/lactose and cytisine succinate/lactose stored for 9 days at 40°C and 75% RH

No.	CP of cytisine by HPLC, area %
1 - Cytisine/ lactose	62.75
2 - Cytisine succinate/lactose	77.47

As can be seen from the data in Table 4, when present in the form of its succinate salt, cytisine is degraded at a substantially lower rate than when present in free base form. Thus, the succinate salt effectively improves the stability of cytisine and facilitates its formulation with compositions comprising lactose.

Claims

1. A succinate salt of cytisine.

2. The salt of Claim 1 wherein the salt is cytisine hydrogen succinate.

3. A solvate or hydrate of the salt of Claim 1 or 2.

4. A pharmaceutical composition comprising the salt of any one of Claims 1 to 3 and a pharmaceutically acceptable excipient.

5. The pharmaceutical composition according to Claim 4, wherein the composition is in the form of a tablet or capsule.

6. The pharmaceutical composition according to Claim 4 or 5 wherein the pharmaceutically acceptable excipient is lactose, corn starch and / or wheat starch

7. The pharmaceutical composition according to any one of Claims 4 to 6 wherein the composition is provided as a unit dosage form, said unit dosage form comprising 1 to 3mg of the salt of any one of Claims 1 to 3.

8. The pharmaceutical composition according to any one of Claims 4 to 7 for use in the treatment of nicotine addiction.

Intellectual Property Office

Application No:	GB1701939.9	**Examiner:**	Mr Robert Goodwill
Claims searched:	1-8	**Date of search:**	8 September 2017

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	CN 104710422 A (SHANGHAI YIZHI MEDICINE TECHNOLOGY), see EPO abstract AN CN-201310684148-A
A	-	US 2010/048606 A1 (KOZIKOWSKI), see paragraphs [0015] and [0382]
A,&	-	WO 2015/025718 A1 (HISAMITSU PHARMACEUTICAL CO)
P,&	-	US 2016/199315 A1 (TAKAGI), see paragraphs [0020], [0021] and [0088]

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[X] :

Worldwide search of patent documents classified in the following areas of the IPC

A61K; A61P; C07D

The following online and other databases have been used in the preparation of this search report

BIOSIS, CAS-ONLINE, EPODOC, MEDLINE, WPI, XPESP, XPSPRNG

International Classification:

Subclass	Subgroup	Valid From
C07D	0471/18	01/01/2006
A61K	0031/439	01/01/2006
A61P	0025/34	01/01/2006