IQB-9302.HCl

DMF

1. APPLICANTS PART

PROT. NO.: DMF 9454/01
DATE: 15/02/99
Q.A.: Mª LUISA ESPINÓS

CONFIDENTIAL INFORMATION

TABLE OF CONTENTS

MANUFACTURER

Company data

1. Name:

   LABORATORIOS ESPINOS Y BOFILL,S.A.
   VAT: ES A08/150.450

2. Headquarter and Chemical Factory:

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   08940 Cornellá - BARCELONA - (Spain)
   Telf. 34+93 377 00 51
   Fax 34+93 377 51 58
   E-mail lebsa@sefes.es

3. Technical Direction:

   Dr. J.Mª. Espinós Tayá

NAMES

- DL-N-METYLCYCLOPROPYL-PYPERIDINE-2-CARBOXYL-2,6-DIMETHYLANILIDE, HYDROCHLORIDE

- I.Q.B.- 9302.HCl

PHYSICAL DATA

Physical data:

- **Physical characteristics:**

A white crystalline powder.

Odourless or practically odourless.

Bitter taste that rapidly disappears because of anaesthesia of oral mucosae.

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- **Solubilities**

Soluble in water and DMSO.

Soluble in hot ethanol

Insoluble in acetone, chloroform, ether and methylene chloride.

- **Melting point**

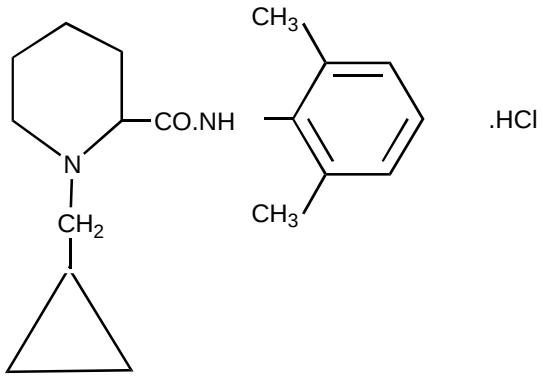
About 260ºC with descomposition

- **Dissociation constant**

pKa = 7.99 ± 0.006

NAMES

1. Structural formula



2. Molecular formula

$C_{18}H_{27}.Cl\ N_2O$

3. Molecular weight

322.5

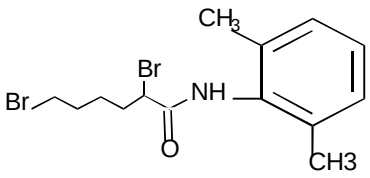
SYNTHESIS REACTION

Reaction Data:

SYMBOL:	CHEMICAL NAME	FORMULA NUMBER	MOLEC. WEIGHT
a	2,6-DIBROMOHEXANOYL-2,6-DIMETHYLANILIDE	9410	377
b	DL-N-METHYLCYCLOPROPYL-PIPERIDIN-2-CARBOXYL--2,6-DIMETHYLANILIDE	9453	286
c	DL-N-METHYLCYCLOPROPYL-PIPERIDIN-2-CARBOXYL--2,6-DIMETHYLANILIDE HYDROCHLORIDE	9454	322.5

SYNTHESIS REACTION

a)



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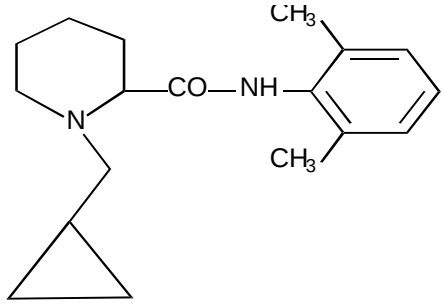

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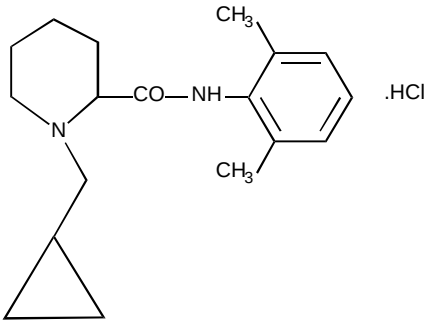
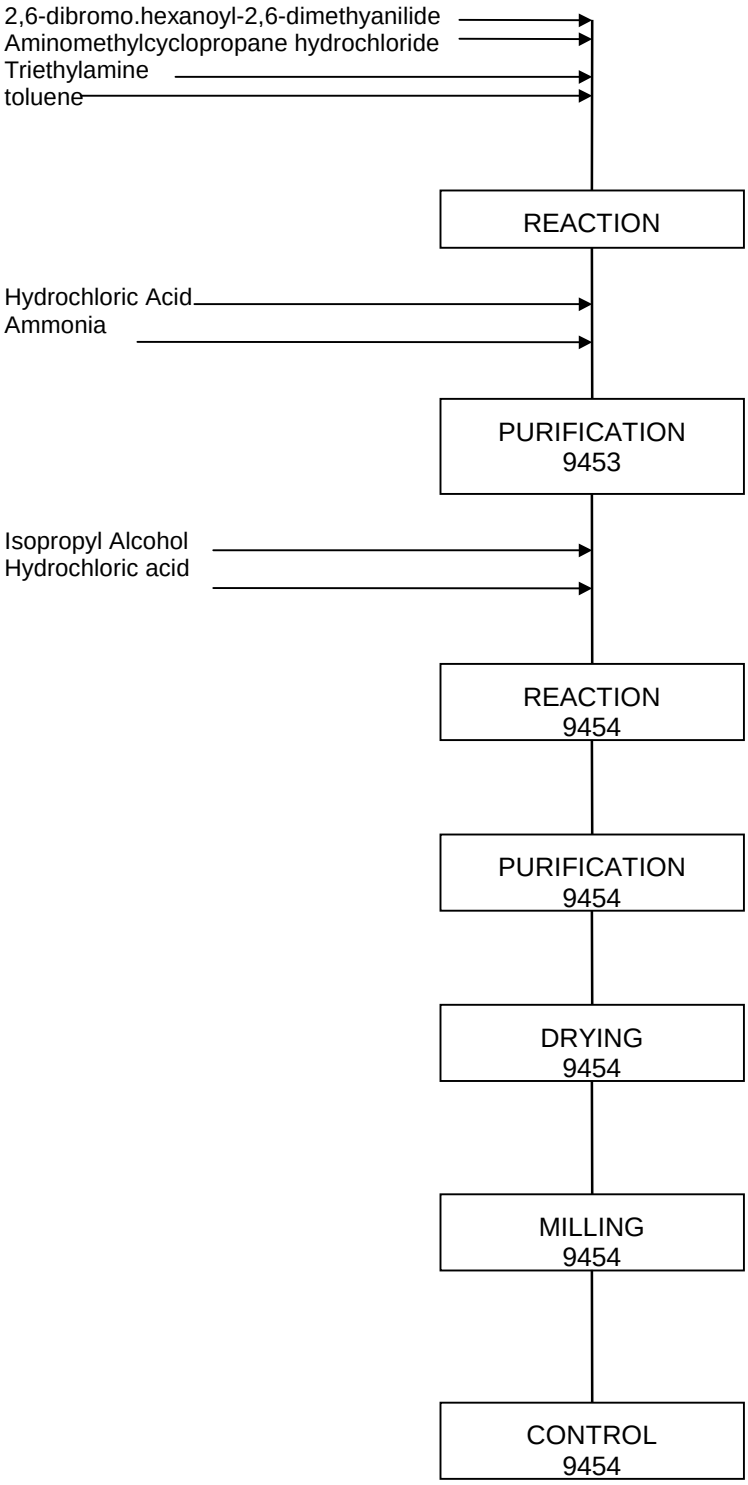


FLOW DIAGRAM: IQB-9302.HCl



FINISHED PRODUCT SPECIFICATIONS COVER AND SPECIFICATION SHEET	SPEC. NO.: FP-002.98.02 EFFECTIVE DATE: 5/02/99 REFERENCE: 211.160 SUPERSEDES: FP-002.98.01 PAGE 1 OF 1 PAGES

PRODUCT NAME:

IQB-9302.HCl

DESCRIPTION OF PRODUCT:

White powder. Soluble in water and DMSO. Insoluble in acetone, chloroform and ether.

PACKAGING REQUIREMENTS:

Paper Kraft drums with two plastic bags.

LABELLING REQUIREMENTS:

Product name, lot number, weight

TEST NAME	TEST METHOD NUMBER	SPECIFICATION
Appearance		White powder
I.R. Spectrum	98/018.01	Similar to standard
Chlorides	98/020.01	To pass test
Color	98/023.01	To pass test
Opalescence	98/024.01	To pass test
Acidity or alkalinity	98/025.01	To pass test
TLC	98/022.02	Max.: 0.25%
% 2,6-dimethylaniline	98/026.01	Max.: 100ppm
Heavy metals	98/027.01	Max.: 10 ppm
Loss on drying	89/052.01	Max.: 1.0%
Ash	98/028.01	Max.: 0.1%
Purity HPLC	98/017.02	Min.: 99.0%
Assay	98/019.01	98.5 - 101.0%
2-propanol	92/117.01	Max.: 0.5%

SPECIAL COMMENTS:

None

AUTHOR: ANNA PONS	DATE: 5/02/99
APPROVED: Mª LUISA ESPINOS	DATE: 5/02/99

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 89/052.01 EFFECTIVE DATE: 4/09/89 REFERENCE: 211.160 SUPERSEDES: A-870201 PAGE 1 OF 1 PAGES

TITLE.

LOSS ON DRYING (105ºC, 24 HOURS)

OBJECTIVES:

DETERMINATION OF LOSS IN DRYING IN OVEN

COMPENDIA REFERENCE:

U.S.P. XXI (731), Page 1249

EQUIPMENT:

OVEN WITH CONTROLLED TEMPERATURE.

CONDITIONS:

OVEN AT 105ºC

PROCEDURE:

Weigh a weighing bottle (A), place about 1 g of sample and weigh (B). Put it in an oven at 105ºC for 24 hours without cover.

Cool the weighing bottle in a dessicator for 15 minutes and weigh again (C).

CALCULATIONS:

$$\% \text{ loss on drying } = \frac{B - C}{B - A} x100$$

AUTHOR: JORDI DOMENECH	DATE: 4/09/89
APPROVED: Mª LUISA ESPINOS	DATE: 4/09/89

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 92/117.01 EFFECTIVE DATE: 10/06/92 REFERENCE: 211.160 SUPERSEDES: 4-1987 PAGE 1 OF 2 PAGES

TITLE.

RESIDUAL ISOPROPANOL

OBJECTIVES:

DETERMINATION OF AMOUNT OF RESIDUAL ISOPROPANOL

COMPENDIA REFERENCE:

EUROPEAN PHARMACOPOEIA - 1992. V.6.20.3

EQUIPMENT:

GAS CHROMATOGRAPHY WITH INTEGRATOR

CONDITIONS:

See the procedure

PROCEDURE:

HP - 5890:

PRECOLUMN: glass with silanized glasswool
COLUMN: 6 ft Tenax GC, 60/80
CONDITIONS: Ti = 110ºC
Ti = 7 min.
Rate = 0ºC/min — OVEN MAX = 225ºc
Tinj = 190ºC — RANGE = 5
Tdet = 190ºC — ATTN = 0

INTEGRATOR: HP-3390

ZERO = 10
ATT = 3 — TIME 0 — INTG 8 (only HP-3393)
CHT. SP. = 0.5 — TIME 7 — STOP
AR REJ = 0
THRSH = 3
PK WD = 0.16

SAMPLE: 10% solution in water.

STANDARD: Put in a 50 ml. Volumetric flask 0.1 ml of isopropanol. Make level with deionized water (Sol.A).Transfer 5 ml of Sol.A in a 100 ml volumetric flask and make level with deionized water.

METHOD:

Pre-set the apparatus (S.O.P. I-0067.).
Inject 5µl of standard (S.O.P. I-0068.) and after 5 µl. of sample.

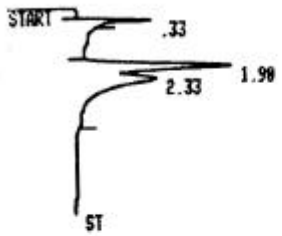
CALCULATIONS:

ppm Isopropanol $= \frac{Am}{Ap} x1000$

A_m = sample peak area

A_p= standard peak area

NOTE: A reference chromatogram is attached.

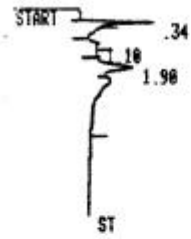


RUN # 10 APR/09/92 11:05:02
ID 51

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.33	63169	PB	0.183	8.425
1.98 →	343210	BV	0.248	45.776
2.33	343370	VB	0.498	45.798

TOTAL AREA= 749750
MUL FACTOR= 1.0000E+00



RUN # 11 APR/09/92 11:17:27
ID 51

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.34	53221	D PB	0.889	34.646
1.10	6109	PB	0.127	3.977
1.98 →	94283	BV	0.274	61.377

TOTAL AREA= 153610
MUL FACTOR= 1.0000E+00

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/017.02 EFFECTIVE DATE: 17/12/98 REFERENCE: 211.160 SUPERSEDES: 98/017.01 PAGE 1 OF 3 PAGES

TITLE.

HPLC OF IQB-9302.HCl

OBJECTIVES:

DETERMINE THE PURITY OF THE SAMPLE

COMPENDIA REFERENCE:

Ph. Eur. 1997 3ºEd. Page 32

EQUIPMENT:

LIQUID-LIQUID CHROMATOGRAPH AND INTEGRATOR

CONDITIONS:

See the procedure

PROCEDURE:

Solutions:

Sample solution: 0.1 g of sample in 5 ml of mobile phase.
Solution 9410: 0.01 g of 9410 in 10 ml of mobile phase.
Solution DMA: 0.02 g of DMA in 10 ml of mobile phase.
Reference solution. 0.1 g of IQB-9302.HCl + 0.5 ml of solution 9410 (0.5%)+ 0.05 ml of solution DMA (0.1%) in 5 ml of mobile phase.

HP-1090:

Column: Hypersil ODS 5µ, 250 x 4.6 mm
Mobile phase: 6.8 g of potassium dihidrogen phosphate in 500 ml of water +500 ml of acetonitrile

Conditions:

FLOW = 1.5
B = 0; C = 0
STOP TIME = 25 min
POST TIME = 0.5
FILTER = 2 (230nm)

TIMETABLE EMPTY

Integrator:

ZERO = 10
ATT 2↑ = 5
CHT SP = 0.5
AR REJ = 0
THRSH = 4
PK WD = 0.16

TIME 0 INTG #8
TIME 25 STOP

Method:

Pre-set apparatus (SOP I-0058).
Filter the solutions (SOP I-0056) and inject 5µl of sample solution and reference solution.

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PROCEDURE (CONTINUED):

FOR IQB-9302;

Inject once the reference solution and then the sample solution.

The purity is at least of 95.0%.

****FOR IQB-9302.HCl***:

Inject three times the reference solution.

The coefficient of variation should be less than 2%. Inject twice the sample solution.

The purity is at least of 99.0%.

Calculations:

$$\%Dimethylaniline = \frac{\%\ \text{Area Dimethylaniline}}{7.8}$$

$$\%9410 = \frac{\%\text{Area 9410}}{1.5}$$

% Other impurities = % Area other impurities.

% 9454 = 100 - % Dimethylaniline - % 9410 - % other.

PROCEDURE (CONTINUED):

CHROMATOGRAM OF REFERENCE:

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/018.01 EFFECTIVE DATE: 31/08/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE.

IQB-9302.HCl I.R. SPECTRUM

OBJECTIVES:

IDENTIFICATION OF THE PRODUCT

COMPENDIA REFERENCE:

EUROPEAN PHARMACOPOEIA - 1997. Page 27

EQUIPMENT:

INFRARED SPECTROPHOTOMETER

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

About 10 g of sample are mixed and ground with about 200 mg of pure and dry potassium bromide and the mixture loaded into a die.

When subjected to high pressure a solid and robust glass-like disk is supporting potassium bromide (SOP I-0051).

The disk is mounted in a holder in the spectrophotometer and the spectrum of sample recorded (SOP I-0052).

REFERENCE SPECTRUM:

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/019.01 EFFECTIVE DATE: 31/08/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE.

IQB-9302.HCl TITRATION

OBJECTIVES:

CALCULATE THE CONCENTRATION OF THE SAMPLE.

COMPENDIA REFERENCE:

EQUIPMENT:

POTENTIOMETRIC TITRATOR, BALANCE AND RECORDER.

CONDITIONS:

SEE THE PROCEDURE.

PROCEDURE:

Weight exactly about 0.2 g of sample and dissolve in glacial acetic acid (≈40 ml).

Add 10 ml of mercuric acetate and titrate potentiometrically with 0.1N perchloric acid.

The result should be between 98.5 and 101.0%.

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/020.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

CHLORIDES IN IQB-9302.HCl

OBJECTIVES:

DETERMINATION OF THE CHLORIDES CONTENT.

COMPENDIA REFERENCE:

Ph. Eur. 1997, 2.3.1.(a)

EQUIPMENT:

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

Dissolve 0.02 g of sample in 2 ml of deionized water.

Acidify with 2M nitric acid. Add 0.4 ml of 4.25% silver nitrate. Stir and allow to stand protected from light. A white precipitate should appear.

Centrifuge and wash 3 times with 1 ml of deionized water. Add 2 ml of water to the precipitate and 1.5 ml of 17% ammonia. The precipitate should disappear.

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/022.02 EFFECTIVE DATE: 2/02/99 REFERENCE: 211.160 SUPERSEDES: 98/022.01 PAGE 1 OF 1 PAGES

TITLE:

TLC IQB-9302.HCl

OBJECTIVES:

SEE PURITY OF THE SAMPLE

COMPENDIA REFERENCE:

Ph. Eur. 1997. Page 30

EQUIPMENT:

CHROMATOGRAFIC PLATES, DEVELOPING CHAMBER AND U.V. LIGHT.

CONDITIONS:

SEE THE PROCEDURE.

PROCEDURE:

Plates: Merck Art. 1.05735 or equivalent (5x10 cm).

Eluent: Methanol : ammonia (90:10)

Solutions:

Sample solution: dissolve 0.50 g of sample in 5 ml of methanol.

Reference (a): dissolve 0.025 g of IQB-9302.HCl standard in 5 ml of methanol.

Reference (b): dilute 0.5 ml of Reference (a) with 100 ml of methanol (0.5%).

Reference (c): dissolve 0.25 g of Aminomethylcyclopropane in 10 ml of methanol. Dilute 0.1 ml of this solution to 10 ml with methanol (0.25%).

Deliver 10µl of sample solutions Reference (b) and Reference (c), on the plate and develop over a path of 10 cm. Dry the plate and examine in U.V. light at 254 nm. Spray the plate with ninhidrine solution.

Any secondary spot in sample solution is not more intense than the spot obtained with Reference (b).

Any spot corresponding to aminomethylcyclopropane in the chromatogram obtained with sample solution is not more intense than the spot obtained with Reference (c).

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/023.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

COLOUR TEST FOR IQB-9302.HCl

OBJECTIVES:

ESTIMATION OF COLOURED IMPURITIES

COMPENDIA REFERENCE:

EUROPEAN PHARMACOPOEIA - 1997. 2.2.2. (II)

EQUIPMENT:

NONE

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

Sample solution: dissolve 1.0 g of sample in 50 ml of deionized water.

Solution B: 3.0 ml of yellow solution + 3.0 ml of red solution + 2.4 ml of blue solution + 1.6 ml of hydrochloric acid 10g/l (1%: 2.7 g of 37% hydrochloric acid in 100 ml of water).

Solution B_9: 1.0 ml. of B solution + 99 ml 10 g/l hydrochloric acid.

The sample solution should have the same appearance of water or, not more intensely coloured than reference Solution B_9.

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/024.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

OPALESCENCE

OBJECTIVES:

TO VERIFY THE ABSENCE OF OPALESCENCE

COMPENDIA REFERENCE:

EUROPEAN PHARMACOPOEIA - 1997. Page 2.2.1

EQUIPMENT:

NONE

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

Sample solution: dissolve 1.0 g of sample in 50 ml of deionized water.

Hydrazine sulphate solution: Dissolve 1 g of hydrazine sulphate in water and dilute to 100 ml with the same solvent. Allow to stand for 4 h to 6 h.

Hexamethylenetetramine solution: Dissolve 2.5 g of hexamethylenetetramine in 25 of water in a 100 ml glass-stopered flask.

Primary opalescent suspension: to the solution of hexamethylenetetramine in the flask add 25 ml of hydrazine sulphate solution. Mix and allow to stand for 24 h. this suspension is stable for 2 months.

Standard of opalescence: Dilute 15 ml of the primary opalescence suspension to 1000 ml with water. This suspension is freshly prepared and may be stored for at most 24 h.

Reference I: in a 100 ml glass stopered flask dilute 5 ml of standard to 100 ml with water.

The sample solution should have the appearance of the water and its opalescence should be not more pronounced than that of the Reference I.

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/025.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

ACIDITY OR ALKALINITY OF IQB-9302.HCl

OBJECTIVES:

CHECK THE PRESENCE OF ACIDIC OR BASIC IMPURITIES.

COMPENDIA REFERENCE:

Ph. Eur. 1997, 0541

EQUIPMENT:

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

Dissolve 0.2 g of sample in 10 ml of deionized water previously boiled. Add 0.2 ml of 0.01M sodium hydroxide. The pH is not less than 4.7.

Add 0.4 ml of 0.01M hydrochloric acid, the pH is not greater than 4.7.

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/026.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

2,6- DIMETHYLANILINE

OBJECTIVES:

ASSURE THE ABSENCE OF 2,6-DIMETHYLANILINE IN THE SAMPLE.

COMPENDIA REFERENCE:

Ph. Eur. 1997, Page 0541

EQUIPMENT:

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

Dissolve 0.50 g of sample in methanol and dilute to 10 ml with the same solvent. To 2 ml the solution add 1 ml of a freshly prepared 10g/l solution of dimethylaminobenzaldehid in methanol and 2 ml of glacial acetic acid and allow to stand for 10 minutes.

And yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 2 ml of a 5mg/l solution of 2,6-dimethylaniline in methanol (100ppm).

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/027.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

HEAVY METALS IN IQB-9302.HCl

OBJECTIVES:

ASSURE THE ABSENCE OF HEAVY METALS.

COMPENDIA REFERENCE:

Ph. Eur. 1997 (0541)

EQUIPMENT:

CONDITIONS:

ORDINARY CONDITIONS

PROCEDURE:

Dissolve 2.0 g of sample in 20 ml of water: methanol (15:85). Dilute to 20 ml with the same solvent (solution A). To 12 ml of solution A add 2 ml of buffer solution of acetates pH = 3.5 and add 1.2 ml of thioacetamide reagent. Mix immediately.

Prepare a standard with 10 ml of 1 ppm lead solution and 2 ml of solution A. Add 2 ml of buffer solution and 1.2 ml of thioacetamide reagent.

Finally, prepare a blank with 10 ml of the solvent used (water: methanol), add 2 ml of solution A. Add 2 ml of buffer solution and 1.2 ml of thioacetamide reagent.

After 2 min, any brown colour in the test solution is not more intense than that in the standard.

TEST METHOD PROCEDURE COVER SHEET	T.M.#:.: 98/028.01 EFFECTIVE DATE: 1/09/98 REFERENCE: 211.160 SUPERSEDES: N/A PAGE 1 OF 1 PAGES

TITLE:

ASH

OBJECTIVES:

DETERMINATION OF THE NON-VOLATILE INORGANIC MATERIAL IN THE SAMPLE

COMPENDIA REFERENCE:

Ph. Eur. 2.4.14

EQUIPMENT:

MUFFLE FURNACE, OVEN AND DESICCATOR

CONDITIONS:

SEE THE PROCEDURE

PROCEDURE:

Weigh accurately 0.9-1.1 g substance (weight B) in a suitable crucible that previously has been ignited, cooled and weighed (Weight A).

Moisten the sample with 2 ml of 1M sulphuric acid.

Heat gently until white fumes no longer are evolved, then ignite at 600ºC in a muffle furnace.

Cool the crucible and add several drops of 1M sulphuric acid. Ignite again.

Cool and add several drops of 15.8% ammonium carbonate solution. Ignite, cool and weigh (weight C).

Calculations:

$$\% = \frac{C - A}{B - A} \times 100$$

PROOFS OF STRUCTURE

1. **Infra-red Spectrometry**

The infra-red spectrum of lot 9454.001obtained on a potassium bromide disk presents characteristic transmittance bands assigned as follows:

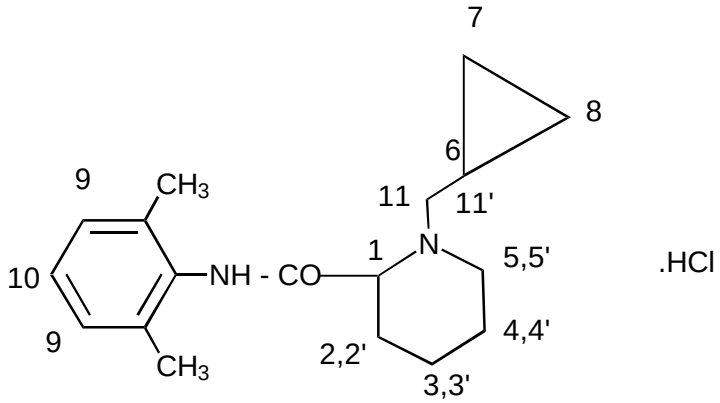
Wavenumber (cm^{-1})	Assignment
3415	NH Stretching.
3110	Aromatic stretching.
2950	Aliphatic stretching.
1673	C=0 Stretching.
1541	Amide II band.
1286	C-N Stretching.

The spectrum was scanned between 4000 and 400 cm^{-1} on a Shimadzu IR—470 dispersive spectrophotometer (see Fig.1).

2. **Proton NMR Spectroscopy**

The ^{1}H-NMR spectra of lot 9454.001 obtained in deuterated Dimethylsulphoxide and deuterated methanol are shown in figures 2 and 3.

The spectrum is assigned according to the structure below:

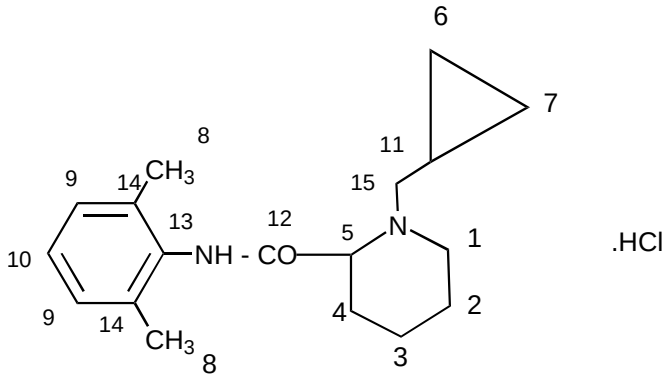


Chemical shift (ppm)	Number of protons	Assignment
4.34	1 (triplet)	H1
2.33	1 (doublet)	H2
1.83	1 (multiplet)	H2’
1.83	1 (multiplet)	H3
1.51	1 (multiplet)	H3’
1.83	1 (multiplet)	H4
1.83	1 (multiplet)	H4’
3.66	1 (doublet)	H5
3.12	1 (multiplet)	H5’
1.18	1 (multiplet)	H6
0.37	2 (multiplet)	H7
0.65	2 (p)	H8
7.09	2 (multiplet)	H9
7.09	1 (multiplet)	H10
3.09	1 (multiplet)	H11
2.91	1 (dd)	H11’
2.14	6 (s)	CH_3
9.90	1	NH
10.7	1(s)	HCl

3. **<u>Carbon-NMR Spectroscopy</u>**

The ^{13}C-NMR spectrum of lot 9454.001 in deuterated chloroform is shown in figure 4.

The spectrum is assigned according to the following structure:



Chemical shift	Assigment
53.13	C1
30.50	C2
23.71	C3
22.55	C4
66.96	C5
5.98	C6
4.46	C7
6.24	C11
18.59	C8
129.39	C9
128.93	C10
168.30	C12
134.12	C13
136.53	C14
62.07	C15

4. **Mass spectrometry**

The Electrospray mass spectrum of lot 9454.001 is shown in figure 5. The assignment of the peaks is listed below:

Peak (m/z)	Fragment
287	mass ion MH^+ $(C_{18}H_{27}N_2O)^+$
138	$[C_9H_{16}N]^+$
84	$[C_5H_{10}N]^+$
55	$[C_4H_7]^+$

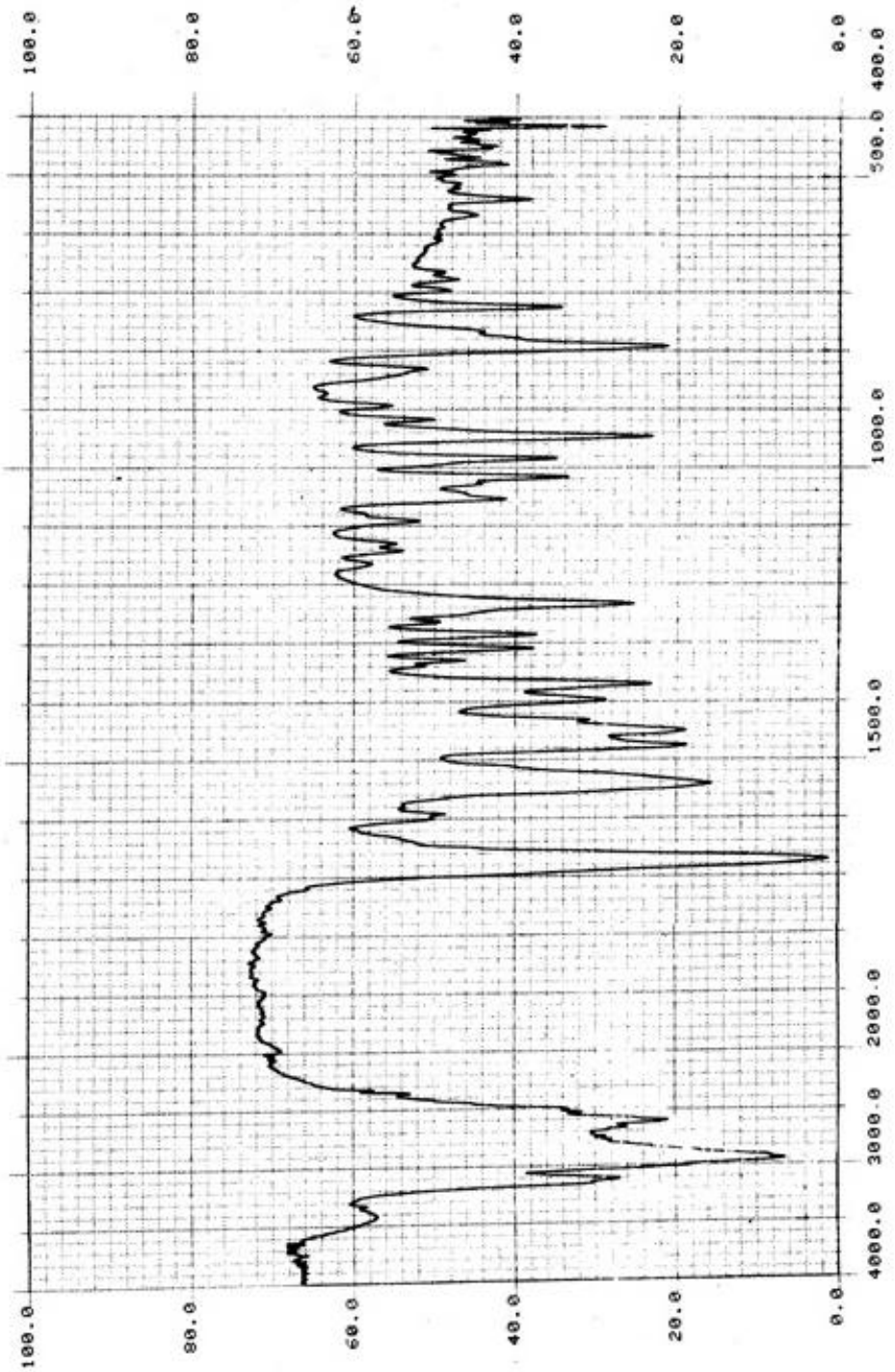
5. **Calorimetric analysis**

The calorimetric analysis confirms that 9454 do not show hydrates or solvates nor polymorphs.

In figures 6 and 7 are attached both the DSC and termogravimetric records.

LEBSA

FIG. 1


100.0
80.0
60.0
40.0
20.0
0.0
400.0
500.0
1000.0
1500.0
2000.0
3000.0
4000.0

LEBSA

FIG. 2

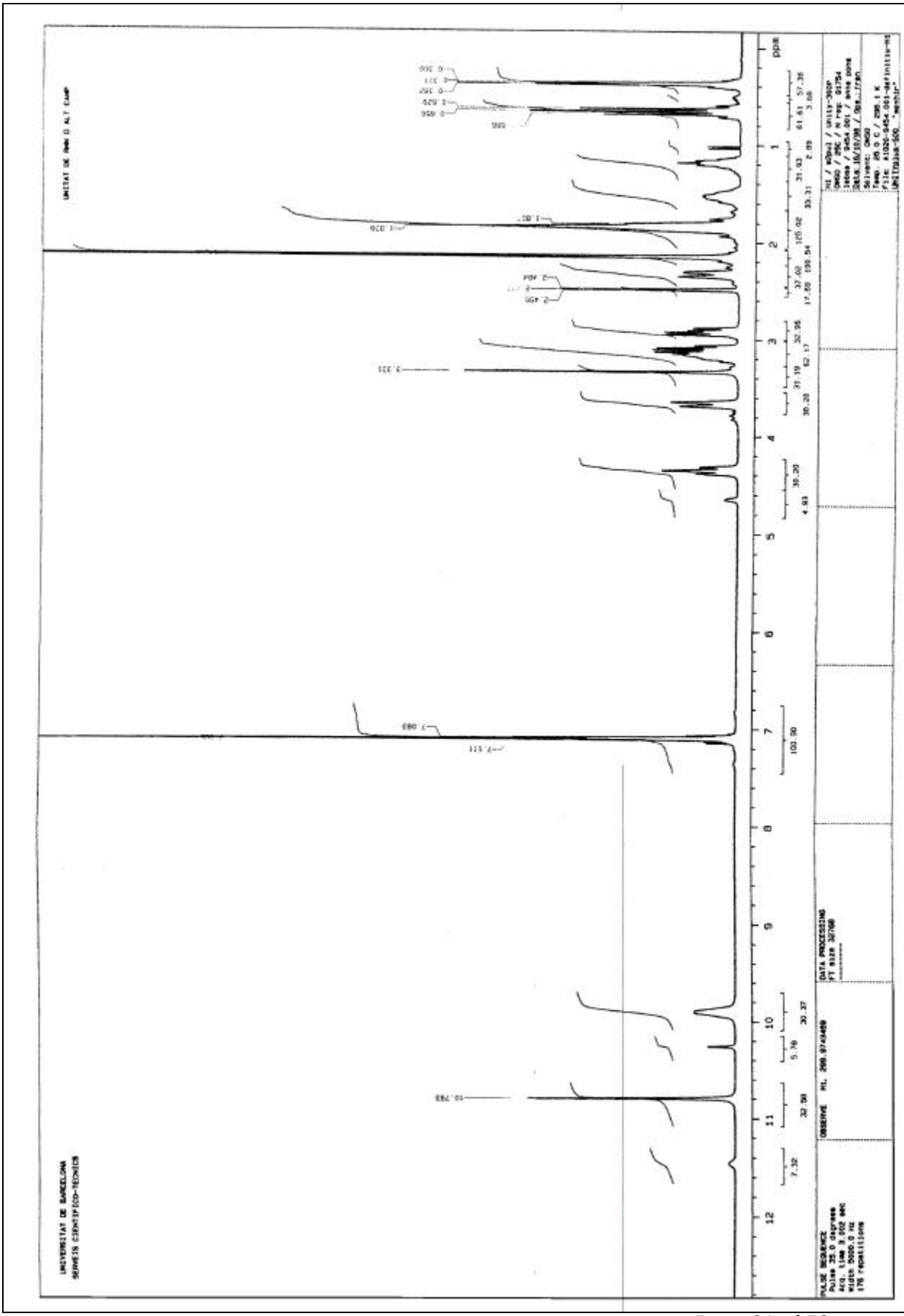


FIG. 3

FIG. 4

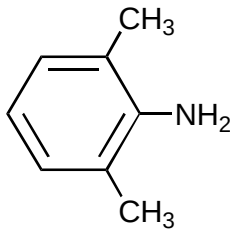
FIG. 5

FIG. 6

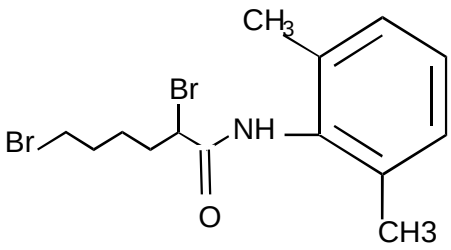
FIG. 7

P O T E N T I A L I M P U R I T I E S

The potential impurities of IQB-9302 are those derived from the synthesis:

1) 

2,6-dimethylaniline

2) 

9410

2,6-dibromhexanoil-2',6'-dimethylanilide.

The HPLC method used for the determination of impurities has been shown to separate these compounds from IQB-9302.HCl #Test method 98/017. Both impurities are available as pure, isolated products.

VALIDATION REPORT: DETERMINATION OF THE ASSAY OF IQB-9302.HCl BY POTENTIOMETRY

1. SUMMARY

1. A validation study was carried out on the potentiometric method for the determination of the assay of IQB-9302.HCl.
2. The precision of the method is as follows:

   Repeatability: C.V. = 0.098%

   Reproductibility: C.V. = 0.060%
3. The linearity of the method is correct over a range of concentrations of 80-120% of IQB-9302.HCl.
4. The accuracy of the method is correct (Recovery = 100.22%).

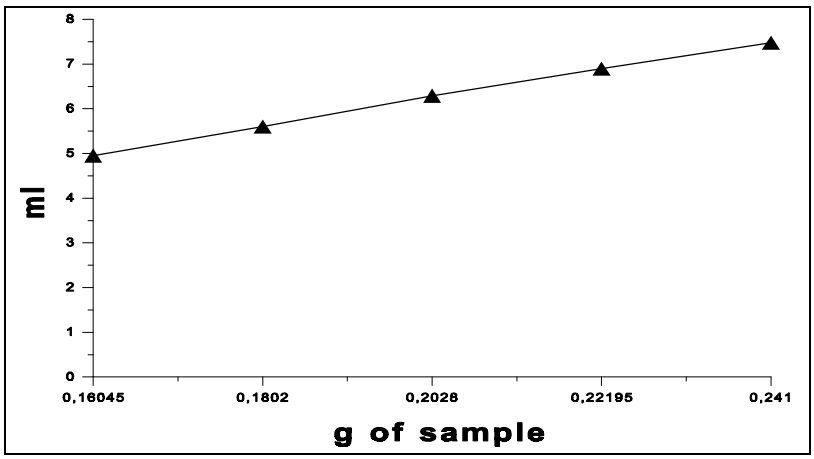
2. SUBSTANTIATION OF VALIDITY

2.1. Linearity

In order to check th linearity of the method, 5 standards of varying concentrations of the sample, were prepared (from 80 to 120% of the normal weight of sample).

	weight of sample (g)	**volume of titrant (ml)**	**f = V/w**
Standard 80%	0.16045	4.9527	30.8676
Standard 90%	0.1802	5.60395	31.0985
Standard 100%	0.2028	6.2921	31.0261
Standard 110%	0.22195	6.8967	31.0732
Standard 120%	0.2410	7.4788	31.0324

$$y = -0.0509 + 31.278\,x$$



r = 0.9999

Response factor: f_m= 31.0196

Standard deviation of the reponse factor: s_f = 0.0900

Coefficient of variation of the response factors: C.V. = 0.2902%

The method is linear over the range 80-120% of the normal amount of analite.

2.2. Precision

a) Repeatability

The same sample of IQB-9302.HCl was prepared and analyzed 10 times to determine the repeatability of the method.

Sample nº	*Result*	*Sample nº*	*Result*
1	100.12%	*6*	100.20%
2	100.02%	*7*	100.28%
3	100.15%	*8*	100.37%
4	100.20%	*9*	100.16%
5	100.31%	*10*	100.36%

Mean = 100.22%

Standard Deviation = 0.1121

Coefficient of Variation = 0.11%

b) Reproductibility

The same sample of IQB-9302.HCl was analyzed by different analysts and different days to evaluate the reproductibility of the method

	DAY 1	DAY 2	DAY 3	DAY 4
ANALYST 1	100.52%	100.81%	100.48%	100.80%
	100.86%	100.57%	100.56%	100.75%
ANALYST 2	100.54%	100.21%	99.91%	100.13%
	100.85%	100.29%	100.01%	100.08%

n = 24

Mean = 100.46%

Standard Deviation = 0.3180

Coefficient of Variation = 0.32%

2.3. Accuracy

The accuracy of the method was checked using data from the test of precision.

Recovery = 100.22%

The accuracy of the test method is correct.

VALIDATION REPORT: DETERMINATION OF PURITY OF IQB-9302.HCl AND IMPURITIES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1. SUMMARY

1. A validation study was carried out on the HPLC method for the determination of the purity of IQB-9302.HCl together with the impurities contents.
2. The potential impurities studied are the following:
   a) 9410: 2,6-dibromhexanoil-2',6'-dimetilanilide
   b) Dimethylanilide
3. The method is capable of separating IQB-9302.HCl from its possible manufacturing impurities described before.
4. The precision of the determination of IQB-9302.HCl is as follows:

   Repeatability: C.V. = 0.098%

   Reproductibility: C.V. = 0.060%
5. The linearities of these three substances are correct over the range of concentrations considered in each case.
6. The accuracy of the method in respect of the determination of the impurities is as follows:

   9410: Recovery = 100.21%

   Dimethylanilide: Recovery = 100.23%
7. The limits of detection of both impurities are assessed to be 0.05% for 9410 and 0.005% for Dimethylanilide.

2. SUBSTANTIATION OF VALIDITY

2.1. Precision

A) Repeatability

A solution of IQB-9302.HCl was prepared and injected 10 times to determine the repeatability of the method. The results were as follows:

99.94%	99.85%
99.93%	99.91%
99.91%	99.87%
99.85%	99.61%
99.91%	99.94%

Mean = 99.87%

Standard Deviation = 0.0981

Coefficient of Variation = 0.098%

b) Reproductibility

Two different analyst analyzed the same sample three different days. The results are summarized in the table below:

	DAY 1	DAY 2	DAY 3
ANALYST 1	99.84%	99.90%	99.99%
	99.88%	99.89%	99.87%
ANALYST 2	99.76%	99.91%	99.95%
	99.89%	99.85%	99.95%

Mean = 99.89%

Standard Deviation = 0.0597

Coefficient of Variation = 0.0598%

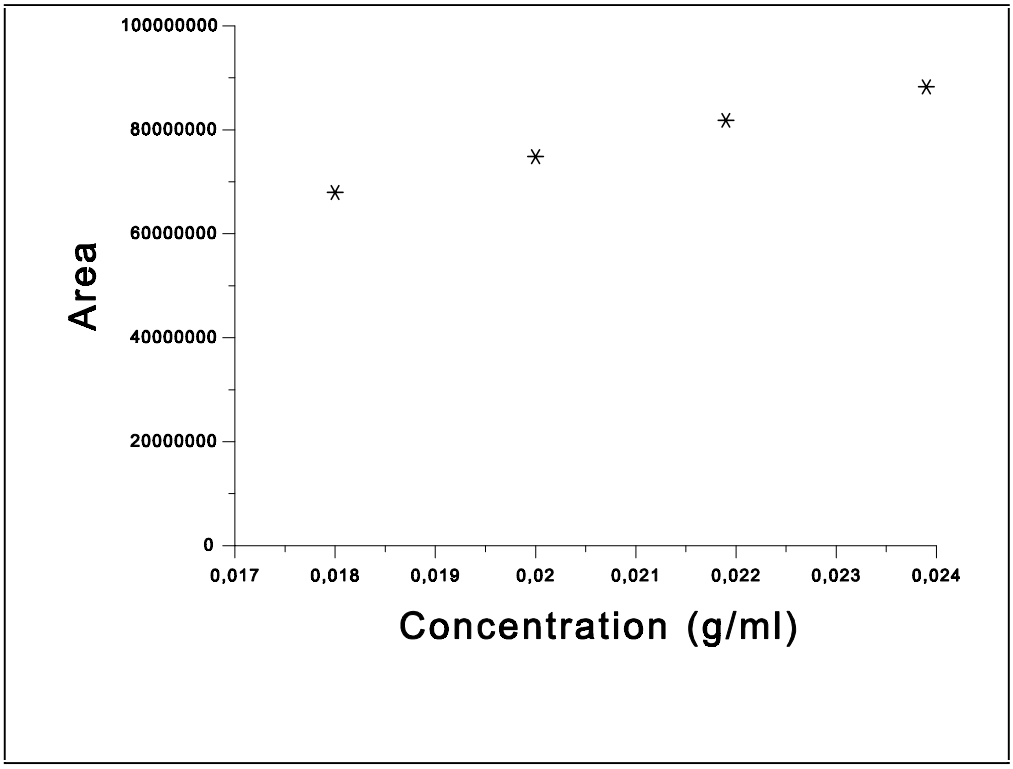
2.2. Linearity

The procedure has been applied to a series of solutions having a concentration range equivalent to 80 - 120% of IQB-9302.HCl content. The results are below:

$$y = 5.077.047 + 3.490.637\ x$$

$$r = 0.9998$$

Concentration (g/ml) (x)	Area (y)	f=Area/Conc.
0.0160	60.836.960	3.802.310
0.0180	67.950.357	3.775.020
0.0200	74.851.285	3.742.564
0.0219	81.833.899	3.736.708
0.0239	88.280.171	3.693.731



Coefficient of variation of the reponse factor: C.V. = 1.096%

The method is linear over the range 80 - 120% of IQB-9302.HCl.

2.3. Especificity

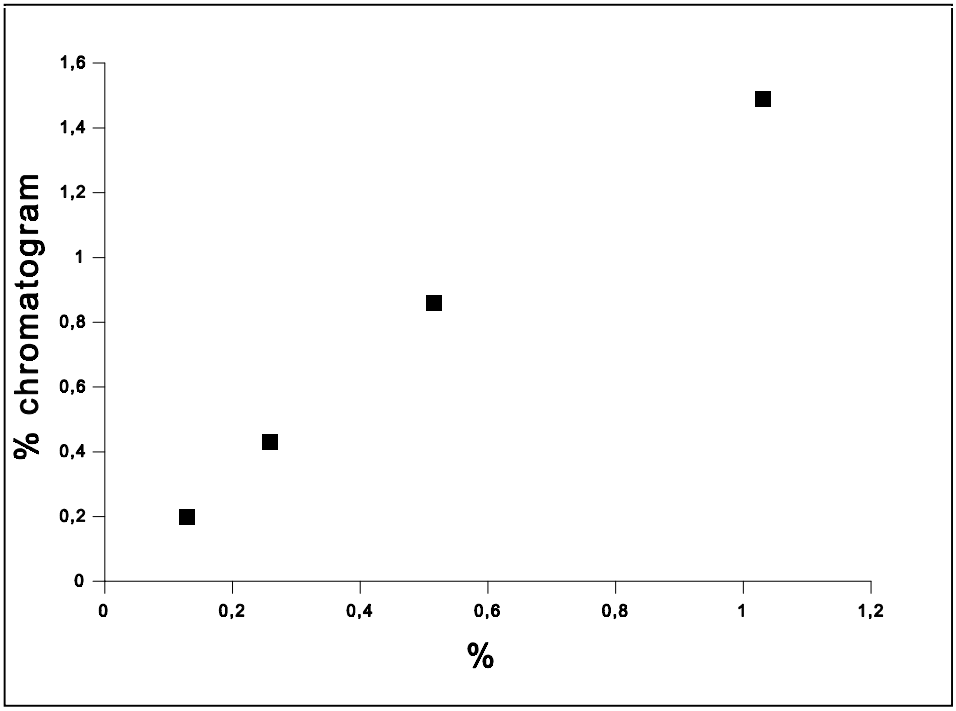
The retention times of the three substances considered, are summarized in the table below:

	Rt (min)	Relative Rt
IQB-9302.HClHCl	2.8	1
Dimetilaniline	4.8	1.7
9410	23	8.2

a) 9410

$$y = 0.072 + 1.403\,x$$
$$r = 0.9968$$

Concentration (%) (x)	Concentration chromatogram (%) (y)	f=Area/Conc.
1.03	1.49	1.45
0.516	0.86	1.67
0.258	0.43	1.67
0.129	0.20	1.55



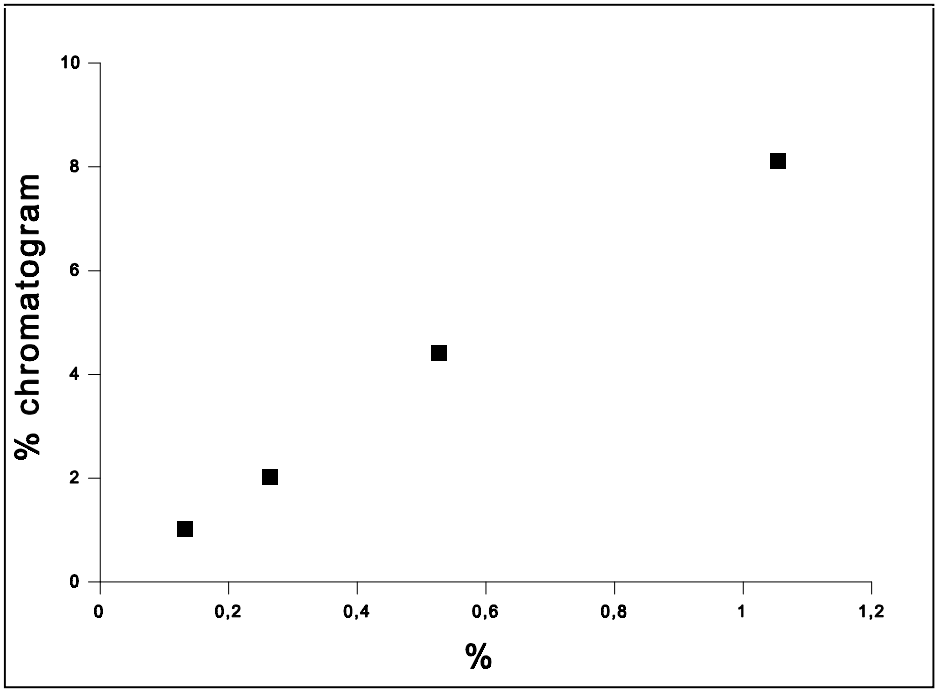
Coefficient of variation of the response factor: C.V. = 6.71%

The method is linear over the range of concentration of 9410.

b) Dimethylanilide

$$y = 0.081 + 7.716\ x$$
$$r = 0.9983$$

Concentration (%) (x)	Concentration chromatogram (%) (y)	f=Area/Conc.
1.054	8.11	7.69
0.527	4.42	8.39
0.264	2.03	7.69
0.132	1.02	7.73



Coefficient of variation of the response factor: C.V. = 4.37%

The method is linear over the range of working concentration.

VALIDATION REPORT: DETERMINATION OF AMINOMETHYLCYCLOPROPANE IN IQB-9302.HCl BY TLC

Summary

1. A validation study was carried out on the thin layer chromatography limit test for the determination of aminomethylcyclopropane in IQB-9302.HCl (test method nº 92/022).
2. The method is capable to separate Aminomethylciclopropane from IQB-9302.HCl. The Rf values are shown in the table below:

	Rf	**RRf**
IQB-9302.HCl	0.61	1
Aminomethylciclopropane	0.35	0.57

3. The limit of detection of Aminomethylcyclopropane is 0.05%.

S T A B I L I T Y S T U D I E S

STABILITY IN STRESSED CONDITIONS

1. **Stability in solution**

The following degradative conditions for IQB-9302.HCl were tested for lot 9454.001:

A1 - 1% solution in water	24 h at room temperature (shaking).
R1 - 1% solution in water	24 h under reflux.
A2 - 1% solution in HCl 0.1N	24 h at room temperature (shaking).
R2 - 1% solution in HCl 0.1N	24 h under reflux.
A3 - 1% solution in NaOH 0.1N	24 h at room temperature (shaking).
R3 - 1% solution in NaOH 0.1N	24 h under reflux.

At the end of times indicated, aliquot of all the obtained solutions was prepared and analyzed using the ordinary conditions. (Test Method no. 98/017).

In general, IQB-9302.HCl has shown a good stability in all conditions. The results are reported in the table below:

Condition	Type of degradation
A1	Unchanged (100%)
R1	Practically unchanged (99.13%)
A2	Unchanged (100%)
R2	Practically unchanged (99.23%)
A3	Unchanged (100%)
R3	Unchanged (100%)

2. **Stability of the solid**

2.1 **In melted state**.

About 1.0 g of IQB-9302.HCl has been kept in the melted state (at 230ºC for 30 minutes). At the end of this time the product is a brown crystal.

The solid obtained has been analyzed by HPLC and it has shown an important degradation:

11.01% IQB-9302.HCl

57.37% 2,6-dimethylanilide.

2.2 **Under U.V. light**

About 1 g of IQB-9302.HCl has been distributed on a thin layer in Petri dishes. The sample has been exposed to the light U.V. (254nm) for 24 hours.

2.3 **Under heat.**

About 1 g of IQB-9302.HCl has been kept at 100ºC for 2 months. After this time, it is observed a partial degradation:

96.77% IQB-9302.HCl

STABILITY IN CURRENT STORAGE CONDITIONS

Examination of samples stored in amber glass bottles at ambient temperature and humidity for up to 5 months has shown that no significant changes were observed.

The ranges of temperature and relative humidity in the warehouse are as follows:

- Range of temperature: 15-25ºC.
- Range of relative humidity: 60-80%.

For the analysis we use the same methods as for the finished product. The analytical controls are done at 3-6-12-24-36-48 and 60 months for the three first batches.

In order to complete this paper, the analytical control has done at 5 months.

On the basis of the results the Quality Control Department establishes the storage precautions and shelf life.

Results are as follows:

BATCH 9454/R.001:

	INITIAL	5 MONTHS
Appearance	White powder	unchanged
Purity HPLC	100.00%	99.99% (*)

9454/R.002:

	INITIAL	5 MONTHS
Appearance	White powder	unchanged
Purity	100.00%	99.82% (*)

9454/R.003:

	INITIAL	5 MONTHS
Appearance	White powder	unchanged
Purity	100.00%	99.73(*)

(*)new HPLC method (T.M. 98/017.02)

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CERTIFICATE OF ANALYSIS

PRODUCT: IQB-9302.HCl

CONTROL #: 9810034

LOT #: 9454.001

DATE: 8th Oct.1998

ANALYTICAL DATA	SPECIFICATIONS	RESULTS
Appearance	White powder	Conforms
Identification		
I.R. Spectrum	Similar to standard	Conforms
Chlorides	To pass test	Conforms
Appearance of solution	Clear and colourless	Conforms
Acidity or alkalinity	To pass test	Conforms
Related substances	Not more than 0.5%	Conforms
2,6-Dimethylaniline	Not more than 100ppm	Conforms
Heavy metals	Not more than 10 ppm	Conforms
Loss on drying	Not more than 1.0%	0.35%
Sulphates ash	Not more than 0.1%	0.04%
Assay	98.5 - 101.0%	101.0%
Residual Isopropanol	Not more than 0.5%	0.23%
HPLC	Not less than 99.0%	99.68%

Silvia Diéguez
Analyst

Anna Pons
Analytical Department Manager

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E-mail: lebsa@sefes.es

CERTIFICATE OF ANALYSIS

PRODUCT: IQB-9302.HCl

CONTROL #: 9812004

LOT #: 9454.002

DATE: 2nd Dec.1998

ANALYTICAL DATA	SPECIFICATIONS	RESULTS
Appearance	White powder	Conforms
Identification		
I.R. Spectrum	Similar to standard	Conforms
Chlorides	To pass test	Conforms
Appearance of solution	Clear and colourless	Conforms
Acidity or alkalinity	To pass test	Conforms
Related substances	Not more than 0.5%	Conforms
2,6-Dimethylaniline	Not more than 100ppm	Conforms
Heavy metals	Not more than 10 ppm	Conforms
Loss on drying	Not more than 1.0%	0.74%
Sulphates ash	Not more than 0.1%	0.00%
Assay	98.5 - 101.0%	99.80%
Residual Isopropanol	Not more than 5000 ppm	3395 ppm
HPLC	Not less than 99.0%	100.00%

Silvia Diéguez
Analyst

Anna Pons
Analytical Department Manager

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CERTIFICATE OF ANALYSIS

PRODUCT: IQB-9302.HCl **CONTROL #:** 9812005

LOT #: 9454.003 **DATE:** 2nd Dec.1998

ANALYTICAL DATA	SPECIFICATIONS	RESULTS
Appearance	White powder	Conforms
Identification		
I.R. Spectrum	Similar to standard	Conforms
Chlorides	To pass test	Conforms
Appearance of solution	Clear and colourless	Conforms
Acidity or alkalinity	To pass test	Conforms
Related substances	Not more than 0.5%	Conforms
2,6-Dimethylaniline	Not more than 100ppm	Conforms
Heavy metals	Not more than 10 ppm	Conforms
Loss on drying	Not more than 1.0%	0.19%
Sulphates ash	Not more than 0.1%	0.01%
Assay	98.5 - 101.0%	99.90%
Residual Isopropanol	Not more than 5000 ppm	3601 ppm
HPLC	Not less than 99.0%	100.00%

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