Presentazione:
Use of coupled chromatographic analytical techniques (HPLC/MS; GC/MS) in the registration of new medicinal products

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Agenzia Italiana del Farmaco
AIFA

Public Declaration of transparency/interests*

The view and opinions expressed are those of the individual presenter and should not be attributed to AIFA

Interests in pharmaceutical industry	NO	Current	From 0 to 3 previous years	Over 3 preavious years
DIRECT INTERESTS:				
1.1 Employment with a company: pharmaceutical company in an executive role	X	☐	☐	☐ mandatory
1.2 Employment with a company: in a lead role in the development of a medicinal product	X	☐	☐	☐ mandatory
1.3 Employment with a company: other activities		☐	☐	X
2. Consultancy for a company	X	☐	☐	☐ optional
3. Strategic advisory role for a company	X	☐	☐	☐ optional
4. Financial interests	X	☐	☐	☐ optional
5. Ownership of a patent	X	☐	☐	☐ optional
INDIRECT INTERESTS:				
6. Principal investigator	X	☐	☐	☐ optional
7. Investigator	X	☐	☐	☐ optional
8. Grant or other funding	X	☐	☐	☐ optional
9. Family members interests	X	☐	☐	☐ optional

Federica Bruno, in accordance with the Conflict of Interest Regulations approved by AIFA Board of Directors (25.03.2015) and published on the Official Journal of 15.05.2015 according to EMA policy /626261/2014 on the handling of the conflicts of interest for scientific committee members and experts.

N.B. I am not receiving any compensation

CONTENT

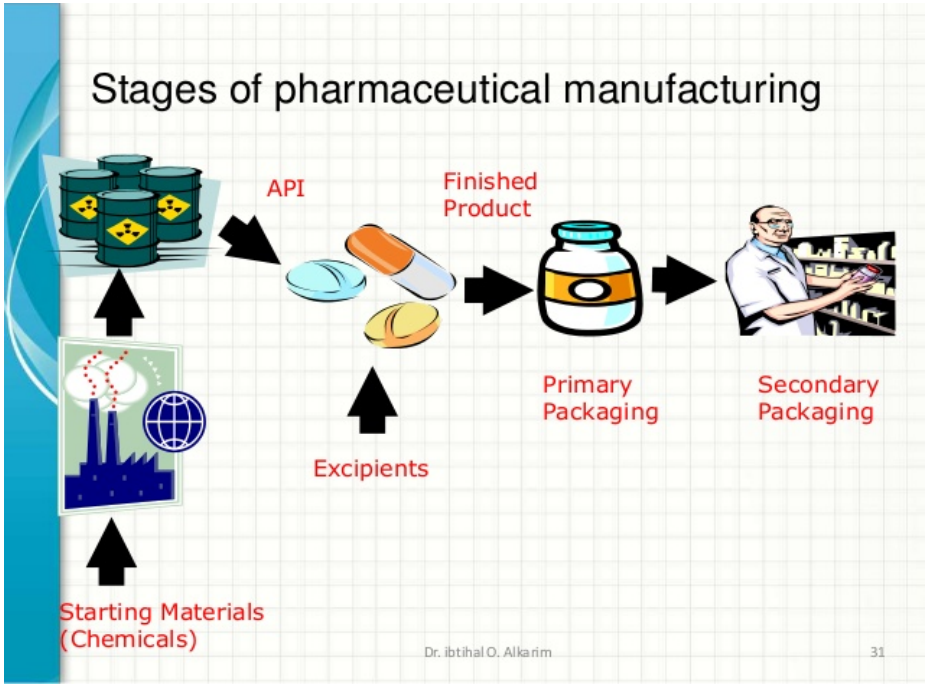
Scope:

The application of hyphenated technologies to support the registration of new pharmaceutical products:

- Introduction
- HPLC-MS & GC-MS
- ICH Guidelines and case studies

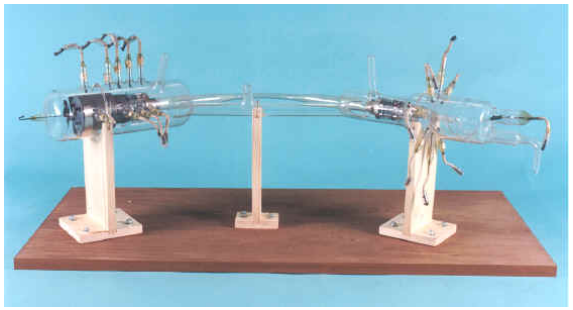
Drug product ... Constituted by ...

- Active substances
- Dosage form
- Formulation
- Manufacturing process
- Packaging



NEW TECHNOLOGIES HELP US!

- LC-MS-MS
- HPLC-DAD-MS
- HPLC-DAD-NMR-MS
- GC-MS
- LC-MS



Application Area

Headspace GC/MSD – volatile organic compound, high migration potential species (e.g. inks, adhesives, glue, processing solvent)

GC-MS – semi-volatile organic compounds, residual monomers, antioxidant, plasticizers, antistatic agents, clarifying agents, preservatives, PAHs, slip agents

LC-UV-MS – non-volatile organic compounds, large oligomers, large antioxidants, thermally labile compounds

Ion chromatography – Bromide, chloride, fluoride, in elastomers

ICP-MS – metals from aluminum canister, glass

Characterization and Identification

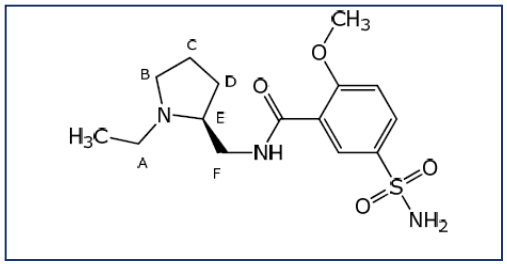
ICH Q6A:

API must be identified to discriminate between compounds of closely related structure which are likely to be present.

The use of two chromatographic procedures is suggested (....) HPLC/UV diode array, HPLC/MS, or GC/MS is generally acceptable.

An example from 3.2.S 3.1 section

Levosulpiride(SulpirideS-enantiomer)

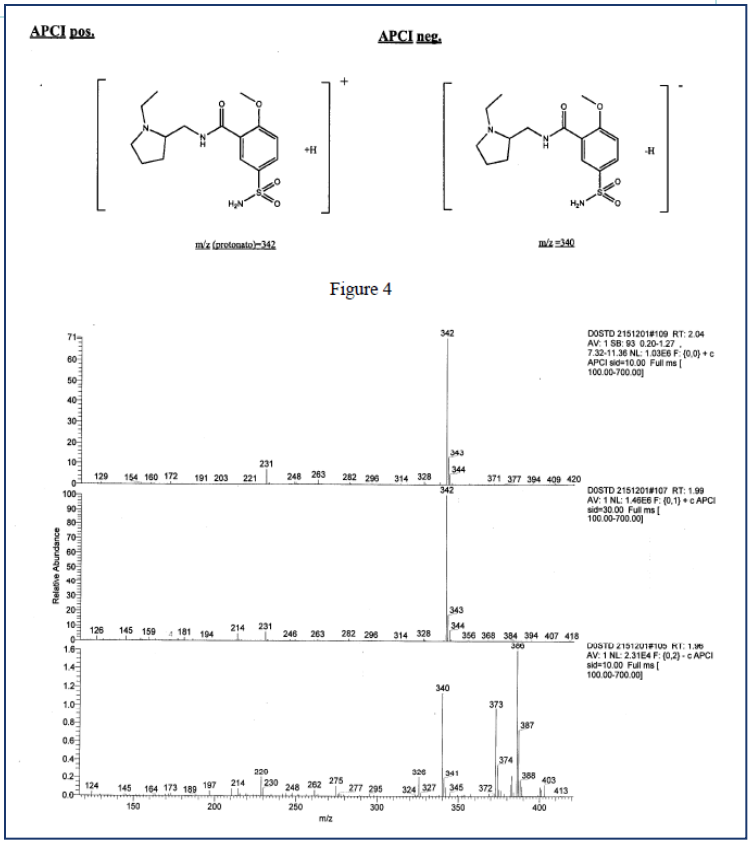


The IR spectrum,

NMR spectra,

<u>mass spectrum: using</u>

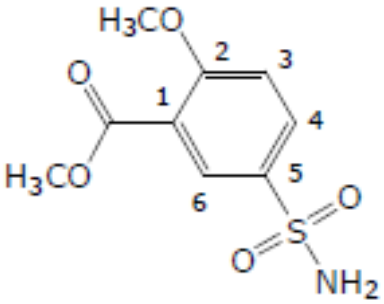
element analysis performed.



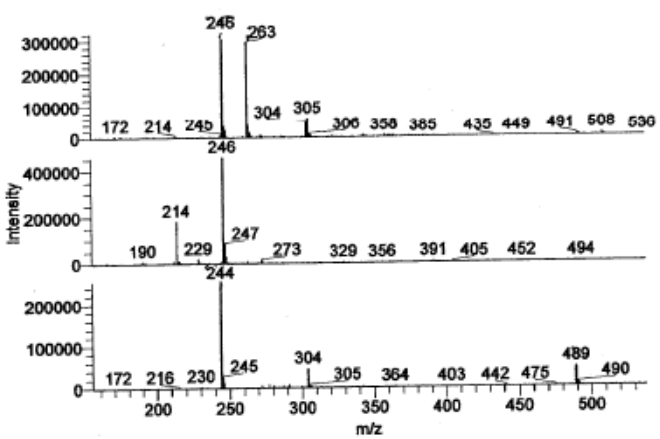
<u>APCI in (+) and (-) mode</u>

The impurities (sect.:3.2.S3.5) :...cont..

2-methoxy-5-sulfamoylbenzoic acid methyl ester



m/z	Attribution
263	$[M+H_2O]^+$ (positive APCI)
246	$[M+1]^+$ (positive APCI)
489	$[M+M-1]^-$ (negative APCI)
244	$[M-1]^-$ (negative APCI)



IMPURITIES and ICH GUIDELINEs

- Indicates the limits to apply for impurities in API and DP
- Impurities and degradation products of pharmaceutical drug substances that exceed the threshold of 0.1% must be identified and qualified by appropriate toxicological studies.
- The presence of a highly toxic impurity must be in the lowest ppm range
- It is a requirement to fully characterize the impurity profile of the active pharmaceutical ingredient and drug product

Cont..

ICH Q6A:

(1) Any component of the new drug substance which is not the chemical entity defined as the new drug substance.

(2) Any component of the drug product which is not the chemical entity defined as the drug substance or an excipient in the drug product."

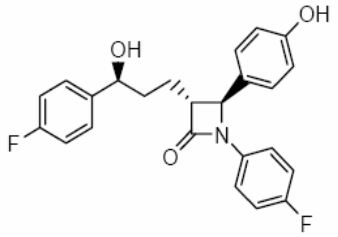
ICH M7:

Mutagenic impurity: An impurity that has been demonstrated to be mutagenic in an appropriate mutagenicity test model, e.g., bacterial mutagenicity assay

EMEA/CHMP/QWP/251344/2006: genotoxic means that there are positive findings in established in vitro or in vivo genotoxicity tests with the main focus on DNA reactive substances that have a potential for direct DNA damage

Ezetimibe in tablets..

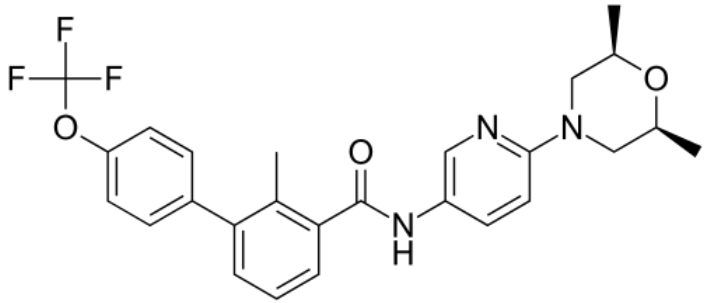
Ezetimibe contains three chiral centers ⇒ eight theoretical potential isomers (four pairs of diastereoisomers).



Q6A ICH states: control of chiral quality for Chiral new API with three or more chiral centers is desirable at a step prior to production of the final drug substance.

- Impurity RRS isomer of Ezitimibe is controlled by a chiral purity method;
- The method is able to distinguish the RRT of the API (SRS), the RRS isomer and the potential Cis-isomers (RRR/SSS or SRR/RSS)
- HPLC-MS analyses applied to confirm the suitability of the proposed method since all the impurities have the same mass and fragmentation

XXX Diphosphate: Syntetic Intermediate Control



Applicant propose the use of GC-MS and HPLC MS for analyzing 2 intermediates.

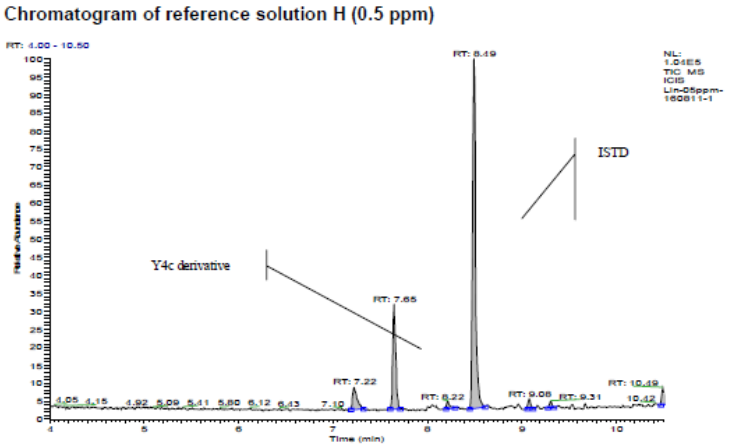
During evaluation of the dossier the Applicant was asked to validate them

cont....XXX- Diphosphate:

GC/MS for intermediate: AAA-Y4c

Limit: Not more than 5 ppm

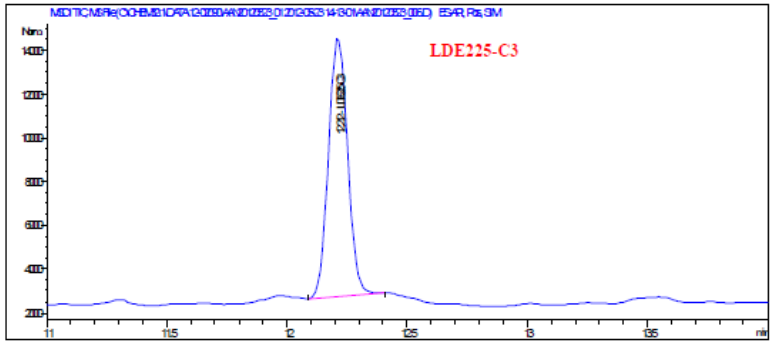
LOD of: 0.2 ppm



LC/MS positive detection for intermediate XYX-Cx

Limit: Not more than 5 ppm

LOQ : 1ppm

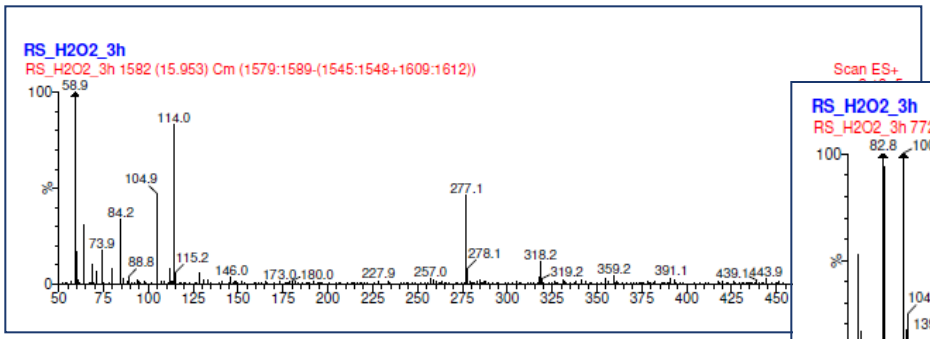


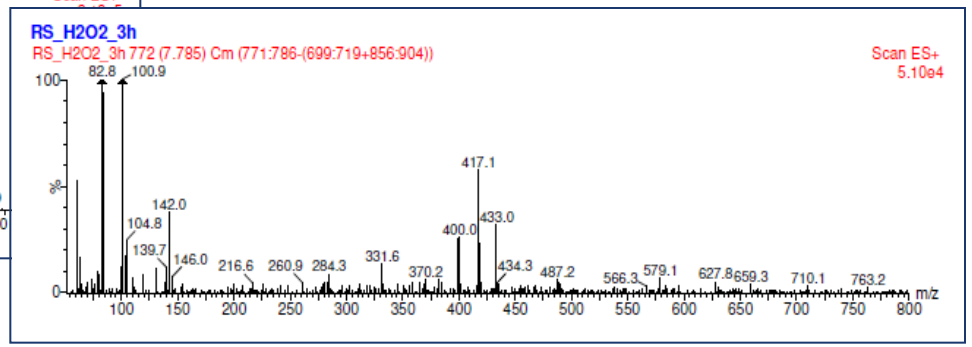
Typical HPLC-MS (SIM) chromatogram of a Reference Solution RS 4 containing LDE225-C3 at a concentration corresponding to 2 ppm; file name aan20120523_006.

Colecalciferol in oral solution: a mass balance issue

Situation: during stability studies a decrease of the assay is evident but the mass balance is complete: it is necessary to demonstrate the reason of the assay decrease.

Applicant: application of the LC/MS techniques allows to identify unknown peaks coming from oxidation reaction whose mass are *m/z+ 401.2, 417.1 and 277.1.*





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Extractables & Leachables: CPMP/QWP/4359/03

- Extraction studies:

required to determine those additives of the material that might be extracted by the preparation or the active substance in contact with the material.

- Migration studies:

should be performed on the active substance/initial formulation to allow the choice of a suitable packaging material for the active substance and the medicinal product

cont...Extractables & Leachables: WHY ?

- Qualification of Container Closure System
- Profile presence of toxic substances (eg. Nitrosamine from rubber
- Extractable limit ensure limitation on leachables when extraxtables are correlated to leachables

Antibody (API) and its container

Container:

4L HDPE Bottle with polypropilene Closure

Study: to enable toxicological and/or product quality assessments an evaluation study of extractables from the container is performed.

Solvent	Description
A	DI H_2O
B	20% ACN, 20% EtOH, 60% H_2O
C	20% ACN, 20% EtOH, 10 mM Ammonium Formate, pH 3, 60% H_2O
D	20% ACN, 20% EtOH, 10 mM Ammonium Formate, pH 7, 60% H_2O
E	20% ACN, 20% EtOH, 10 mM Ammonium Formate, pH 9, 60% H_2O
F	20% ACN, 20% EtOH, 1.0 mM NaCl, 1.0 mM NaH_2PO_4 buffer at pH 3, 60% H_2O
G	20% ACN, 20% EtOH, 1.0 mM NaCl, 1.0 mM NaH_2PO_4 buffer at pH 9, 60% H_2O

The analysis is performed by GC/MS, ICP/MS, HPLC/UV/MS and HPLC/ELSD

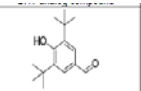
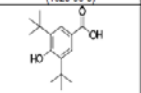
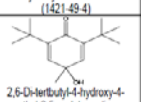
Antibody (API) and its container: Findings

- Inorganic elements (B, Na, Mg, Al, Ca, Si, P, Zn and Br) extracted with solvents A

	Element	HDPE container solvent A
Elements (µg/system)	B	1.3
	Na	18.9
	Mg	8.8
	Al	5.2
	Ca	84.9
	Si	156.5
	P	2.3
	Zn	8.1
	Br	3.8

23 organic substances: organic acids and linear chain ketone and esters, glycols, BHT and BHT's analogs, Irganox 1076 and/or Irganox 1010 degradants,

Irgafos 168 degradants, Nylon degradants

13	 3,5-di-tert-Butyl-4-hydroxybenzaldehyde (1620-98-0)	Reference Standard	GC/MS HPLC/UV LC/MS	A-G	≤265
14	 3,5-Di-tert-butyl-4-hydroxybenzoic acid (1421-49-4)	Reference Standard	HPLC/UV LC/MS	A-G	≤ 40.9
15	 2,6-Di-tertbutyl-4-hydroxy-4-methyl-2,5-cyclohexadiene (10396-80-2)	Reference Standard	GC/MS HPLC/UV LC/MS	B-G	≤ 534

XYX-abc chloride and the Stopper

Drug product: solution for injection, consists of an aqueous solution of XYZ-abc chloride containing sodium chloride, sodium citrate and calcium.

Packaging:10 mL glass vial which is closed by a bromobutyl stopper

Extraction media used:

1. Water for injection (WFI)
2. Isopropanol
3. Thermal extraction without solvent

Extractables found in the aqueous extracts exhibits the highest risk to migrate into the drug product

The detected extractables were used as the basis for the selection of the target substances in a subsequent migration study

XYX-abc chloride .. Cont..

For analysis of the drug product the following validated test methods were used:

- HS-GC Method for volatile substances
- GC-MS Method I for semi volatile substances
- GC-MS Method II for fatty acids
- LC-MS Method for non-volatiles

CONCLUSION:

migration study shows that no leachable compound was detected above the AET in samples of three different lots of sodium citrate solution simulating the drug product solution stored in contact with the bromobutyl stopper.

What about European Pharmacopeia?

LC-MS-MS, LC-NMR, LCN-MR-MS, GC-MS, and LC-MS are going to be included in EP monograph

The state of the art

PEPTIDE MAPPING:

general monograph 2.2.55

NORFLURANE (2257):

MS for identification and GC-MS for test of related substances

What about European Pharmacopeia?

GC-MS methods in Ph.Eur.

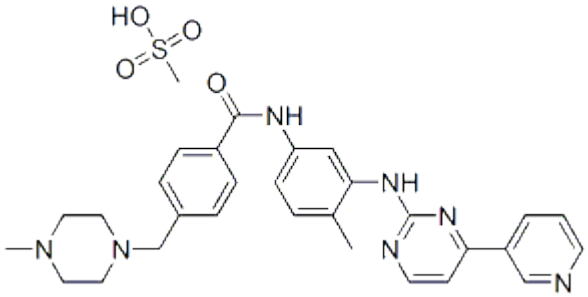
- 2.5.37, Methyl ethyl and isopropyl methanesulfonate in ethanesulfonic acid
- 2.5.38, Methyl ethyl and isopropyl methanesulfonate in active substances
- 2.5.39, Methanesulfonyl chloride in methanesulfonic acid
- 2.5.40, Methyl ethyl and isopropyl toluenesulfonate in active substances
- 2.5.41, Methyl ethyl and isopropyl benzenesulfonate in active substances

BETAHISTINE MESILATE Monograph- 04/2016:1071

PRODUCTION Paragraph:

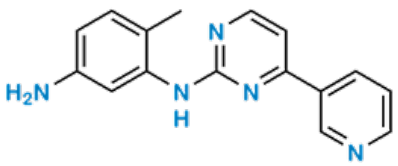
It is considered that alkyl methanesulfonat esters are genotoxic and are potential impurities in betahistine mesilate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation. The general methods 2.5.37. *Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid*, 2.5.38. *Methyl, ethyl and isopropyl methanesulfonate in active substances* and 2.5.39. *Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers

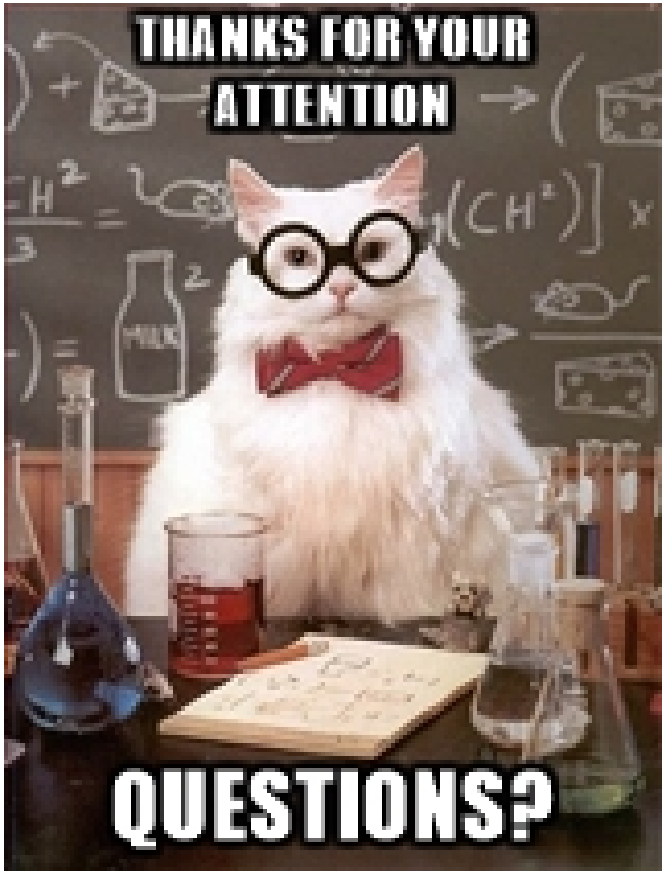
Imatinib mesilate monograph: 2736



Impurity F

- Liquid chromatography (2.2.29) coupled with mass spectrometry (2.2.43).
- *limit*: maximum 20 ppm.





CONTATTI

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