3.2.S.2.3 Control of Materials

3.2.S.2.3.1 List of Materials

The materials used in the manufacturing of Irbesartan API are summarized in Table 3.2.S.2.3-1. Detailed information on materials used in the manufacturing process can be referenced in Section *3.2.S.2.2 Description of Manufacturing Process and Process Control* of this dossier.

Table 3.2.S.2.3-1 List of Materials

No.	Material	Code	Grade	Type
1	BDS	Y743	Industrial	Starting Material
2	BBTT	Y744	Industrial	
3	Toluene	Fresh: Y049 Recovered: RY049	Industrial	Solvent
4	Ethanol	Y050	Industrial	
5	Potable water	EX-004	Industrial	
6	Tetrabutylammonium Hydrogen Sulfate	Y742	Industrial	Auxiliary material
7	Sodium hydroxide	Y030	Industrial	
8	Hydrochloride acid	Y025	Industrial	
9	Activated charcoal	Y064	Pharmaceutical	

3.2.S.2.3.2 Control of the Starting Material

1. Preparation of the starting materials

BDS and BBTT are used as the starting materials in the manufacturing of irbesartan. They are supplied by Henan Huashang Pharmaceutical Co., Ltd. The synthesis routes of BDS and BBTT are presented in Fig 3.2.S.2.3-1 & 2, respectively.

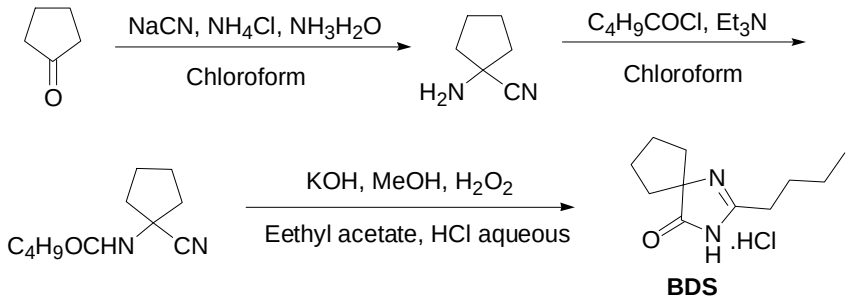


Fig 3.2.S.2.3-1 The Synthetic Route of BDS

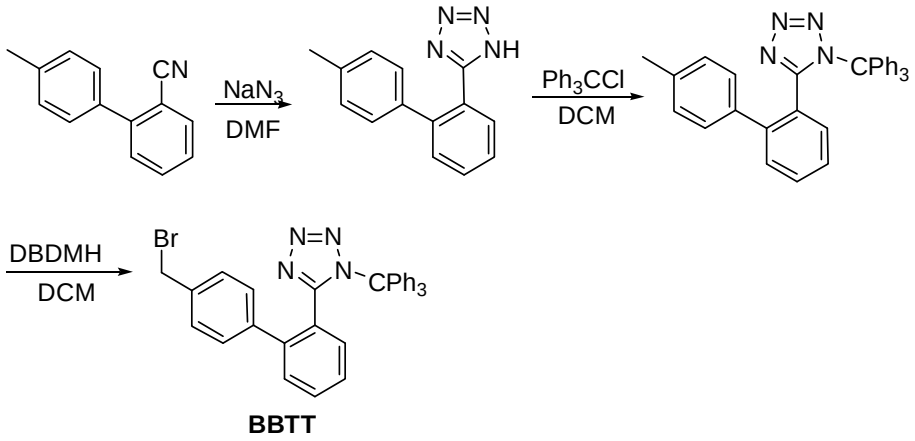
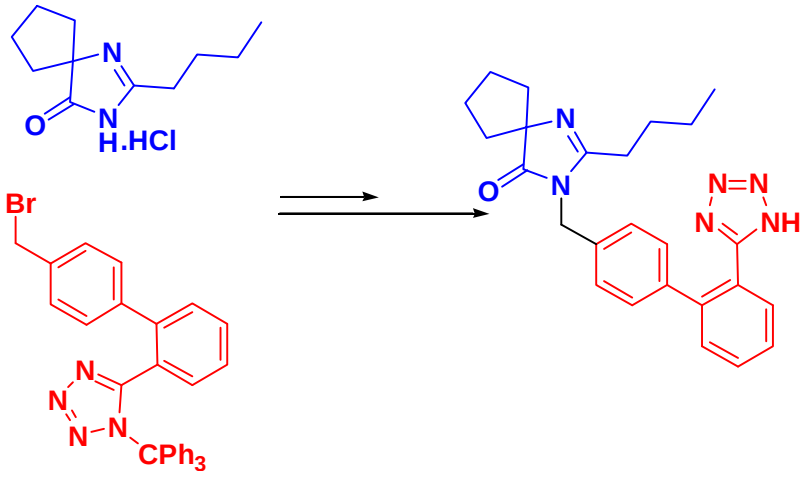


Fig 3.2.S.2.3-2 The Synthetic Route of BBTT

2. Justification of the starting materials

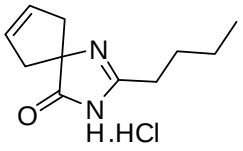
BDS and BBTT are defined as the starting materials because:

1. Then are incorporated into irbesartan as two important structural elements. They contribute significantly to the overall chemical structure of the final drug substance.

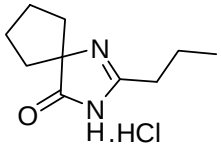


2. They have been widely used in the pharmaceutical industry. BDS is an important material for irbesartan while BBTT is for sartan class drug, both are commercially available in large quantities from multiple vendors.
3. They are synthetic molecules whose chemical structures are well characterized in the literature.
4. The specifications for BDS and BBTT are well defined for their intended use in this manufacturing process based on the specification provide by vendor, who fully ensures its quality. All the test methods used in the specification are normal, well-documented methods which are within the scope of the equipment in Changjiang Pharm.

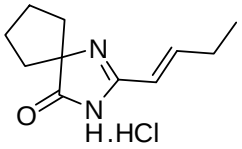
5. The impurity profiles of BDS and BBTT are well documented. The structures and names of the main impurities are provided below:



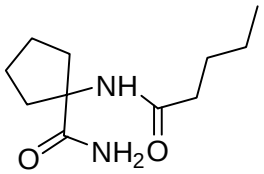
Impurity 0-1



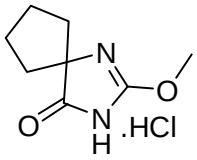
Impurity 0-2



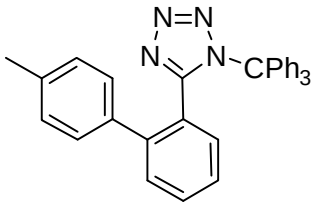
Impurity 0-3



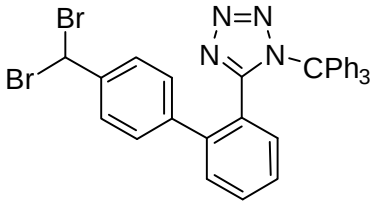
Impurity 0-4



Impurity 0-5



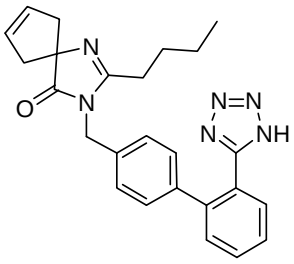
Impurity 0-6



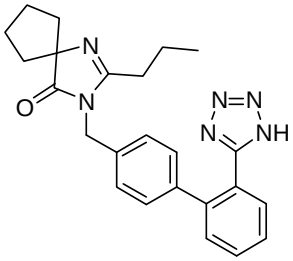
Impurity 0-7

azide

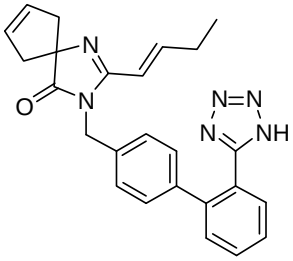
All the above impurities in starting materials can take part in the subsequent reactions and transfer to the corresponding impurities described below.



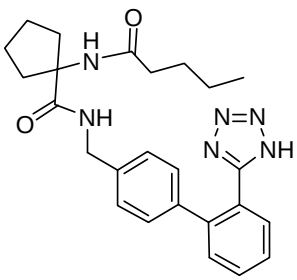
impurty 2-1



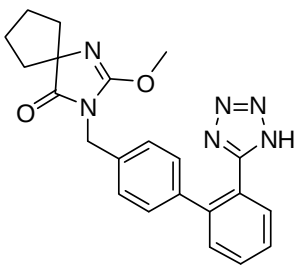
impurty 2-2



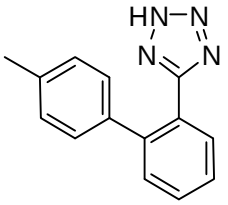
impurity 1



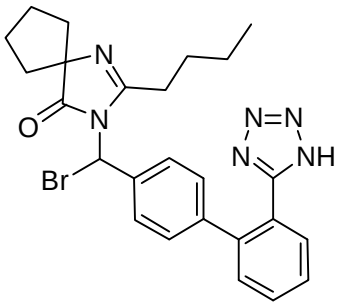
impurity A



impurity 3



impurity 2-6



impurty 2-7

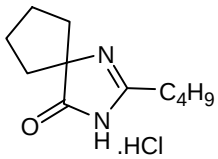
Impurity B: azide

All these eight impurities can dissolve in toluene and ethanol. Irbesartan is obtained with three chemical steps and a purification process. Toluene is used in the purification of IRB02 in which most of the above impurities are eliminated, and the final product is purified in ethanol which further ensures the elimination of these impurities. Impurity A and Impurity B may exist in the final product, however, their contents are strictly controlled as their limits have been defined in the specification of the final product. Detail information on these impurities can be found in section *3.2.S.3.2.2 Organic Impurities* of this dossier.

3. Specification and analytical procedures for BDS

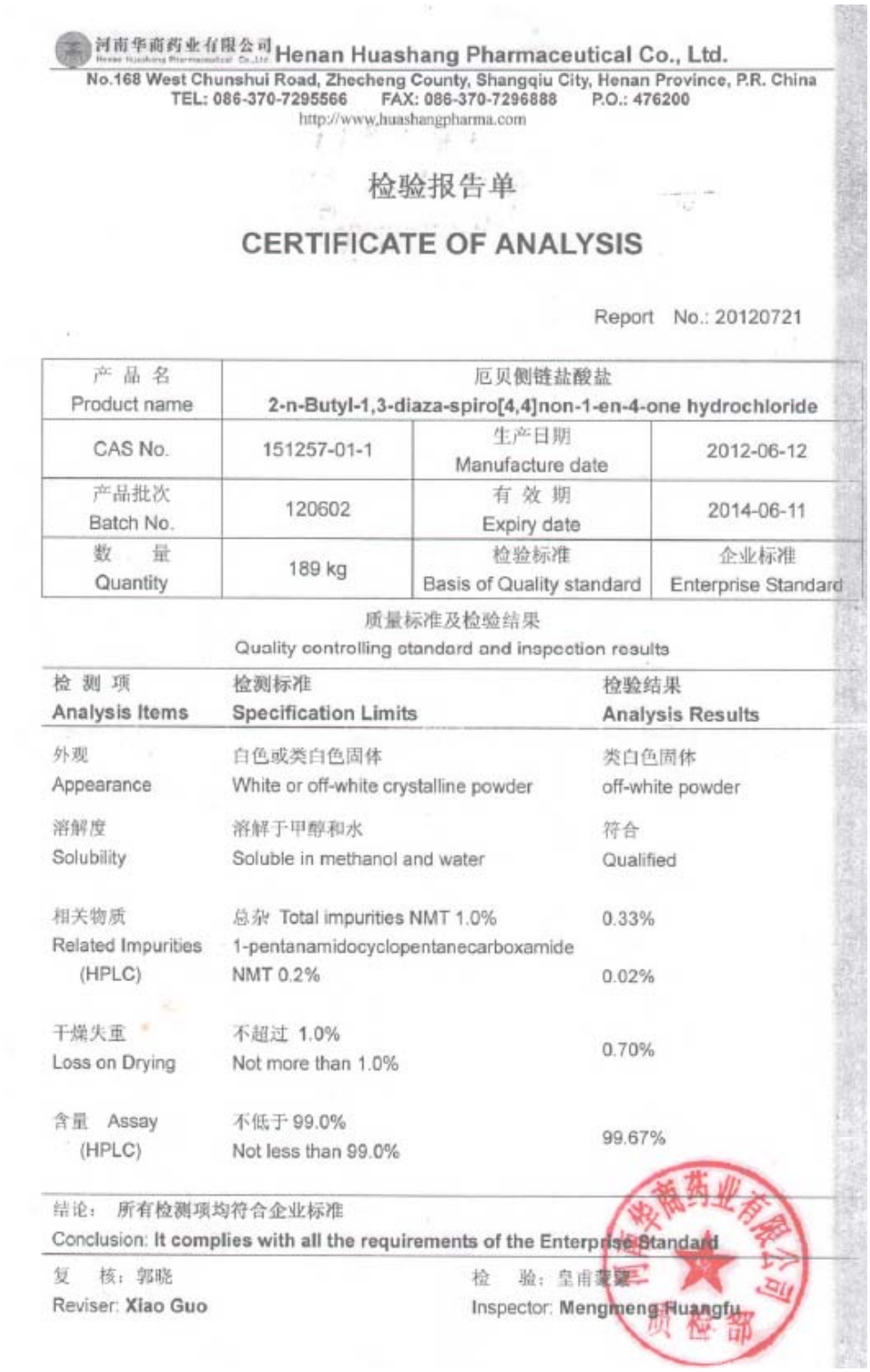
Information on the specification of BDS and the current qualified supplier is provided in Table 3.2.S.2.3-2.

Table 3.2.S.2.3-2 Specification for BDS

Supplier: Henan Huashang Pharmaceutical Co., Ltd.		**Mol. Formula:** $C_{11}H_{19}ClN_2O$ **Molecular Weight:** 230.73
Tests	**Acceptance Criteria**	**Analytical Procedure**
Appearance	White or light-yellow crystalline powder	Visual examination
Identification	The retention time of the major peak in the chromatogram of the Purity preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Purity.	HPLC
Loss on drying	Not more than 1.0%	Drying
Residue on ignition	Not more than 1.0%	Weighing
Related substances – Any impurity	Not more than 0.2%	HPLC
Related substances – Total impurities	Not more than 1.0%	
Purity	Not less than 99.0%	

BDS is accepted based on a certificate of analysis (COA) from the supplier and on meeting internal quality standards following in-house tests.

A representative COA received from the current supplier of BDS, as well as a COA based on in-house testing results provided in Fig 3.2.S.2.3-3 and Fig 3.2.S.2.3-4 respectively. An English translation of the test results is provided followed.



河南华商药业有限公司 Henan Huashang Pharmaceutical Co., Ltd.
No.168 West Chunshui Road, Zhecheng County, Shangqiu City, Henan Province, P.R. China
TEL: 086-370-7295566 FAX: 086-370-7296888 P.O.: 476200
http://www.huashangpharma.com

检验报告单

CERTIFICATE OF ANALYSIS

Report No.: 20120721

产品名 Product name	厄贝侧链盐酸盐 2-n-Butyl-1,3-diaza-spiro[4,4]non-1-en-4-one hydrochloride		
CAS No.	151257-01-1	生产日期 Manufacture date	2012-06-12
产品批次 Batch No.	120602	有效期 Expiry date	2014-06-11
数量 Quantity	189 kg	检验标准 Basis of Quality standard	企业标准 Enterprise Standard

质量标准及检验结果
Quality controlling standard and inspection results

检测项 Analysis Items	检测标准 Specification Limits	检验结果 Analysis Results
外观 Appearance	白色或类白色固体 White or off-white crystalline powder	类白色固体 off-white powder
溶解度 Solubility	溶解于甲醇和水 Soluble in methanol and water	符合 Qualified
相关物质 Related Impurities (HPLC)	总杂 Total impurities NMT 1.0% 1-pentanamidocyclopentanecarboxamide NMT 0.2%	0.33% 0.02%
干燥失重 Loss on Drying	不超过 1.0% Not more than 1.0%	0.70%
含量 Assay (HPLC)	不低于 99.0% Not less than 99.0%	99.67%

结论： 所有检测项均符合企业标准
Conclusion: It complies with all the requirements of the Enterprise Standard

复 核：郭晓 Reviser: Xiao Guo
检 验：皇甫蒙蒙 Inspector: Mengmeng Huangfu

Fig 3.2.S.2.3-3 A Representative COA of BDS Provided by the Current Supplier

文件编号：TS-Y-A184　　版本号：01　　宜昌长江药业有限公司
记录编号：TS-Y-A184a　　Yichang Changjiang Pharmaceutical CO.,LTD

宜昌长江药业有限公司 检验专用章

2-丁基-1,3-二氮杂螺环[4,4]壬-1-烯-4-酮盐酸盐(Y743)检验报告书

Certificate of Analysis for 2-Butyl-1,3-diazaspiro[4,4]non-1-en-one Hydrochloride（Y743）

内部批号	Y743-120801	检验单号	Y_2-1208002
物料来源	河南华商药业有限公司	供应商批号	120602
送检时间	2012 年 08 月 01 日	请验部门	长江药业原料仓库(2)
报告时间	2012 年 08 月 03 日	请验数量	189kg
检验依据	2-丁基-1,3-二氮杂螺环[4,4]壬-1-烯-4-酮盐酸盐质量标准（TS-Y-A184 ，01 版）		

检验项目	标准规定	检验结果
【外观】	应为白色或浅黄色晶体粉末	白色晶体粉末
【鉴别】	供试品溶液主峰保留时间应与对照品溶液主峰保留时间一致	符合规定
【干燥失重】	减失重量应不得过 1.0%	0.37%
【炽灼残渣】	应不得过 1.0%	0.18%
【有关物质】	最大单杂应不得过 0.2%	0.09%
	总杂应不得过 1.0%	0.17%
【纯度】	应不得少于 99.0%	99.8%

结论：本品检测结果符合 TS-Y-A184 ，01 版 规定。

批准人：2012.08.03　审核人：2012.08.03　打印人：2012.08.03

Fig 3.2.S.2.3-4 A Representative COA of BDS issued by Changjiang Pharm

An English Translation of COA for BDS issued by Changjiang Pharm is showed in the following page

Document No.: TS-Y-A184 Version: 01
Record No.: TS-Y-A184a Yichang Changjiang Pharmaceutical CO., LTD

Certificate of Analysis for 2-butyl-1, 3-diazapira [4,4] non-1-en-one hydrochloride

Internal Batch No.	Y743-120801	**Test Sheet No.**	Y_2-1208002
Manufacturer	Henan Huashang Pharmaceutical Co., Ltd.	**Supplier Batch No.**	120602
Sampling date	01-AUG-2012	**Sampling Dept.**	API Warehouse (2)
Reporting date	03-AUG-2012	**Quantity**	189 kg
Standard	Quality Standard of BDS (TS-Y-A184, version: 01)		

Test	**Acceptance criteria**	**Results**
Appearance	White or light-yellow crystalline powder	White crystalline powder
Identification	The retention time of the major peak in the chromatogram of the Purity preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Purity.	Conforms
Loss on drying	Not more than 1.0%	0.37%
Residue on ignition	Not more than 1.0%	0.18%
Related substances	Any impurity: Not more than 0.2% Total impurities: Not more than 1.0%	0.09% 0.17%
Purity	Not less than 99.0%	99.8%

Approved by: Haiyan Zhang 03-AUG-2012 Reviewed by: Yanhong Lan 03-AUG-2012 Printed by: Min Yao 03-AUG-2012

Analytical Procedures

Appearance

Visually examine the material and record the color and physical state. It should be white or light-yellow crystalline powder.

Identification

Proceed as directed in *Purity*. The retention time of the sample peak in the chromatogram corresponds to that obtained with the Standard Solution.

Loss on drying

Accurately weigh 1 g of the sample and place in a weighing bottle which has been dried at 105 °C to constant weight. Heat gradually to 105°C and dry at this temperature until constant weight is obtained.

Calculation:

$$X = \frac{W_1 + W_2 - W_3}{W_1} \times 100\%$$

In which,
X: Loss on drying, %;
W_1: Weight of sample before drying, g;
W_2: Weight of empty weighing bottle after drying at 105 °C, g;
W_3: Weight of weighing bottle and sample after drying at 105 °C, g;

Test two samples in parallel and use the arithmetical mean of the two results as the final result. If one test result fails to meet the acceptance criterion, the result will be unqualified.

Acceptance criterion: The loss on drying should be not more than 1.0%.

Residue on Ignition

Accurately weigh 1 g of the sample and place in a crucible which has been dried at to constant weight. Heat gently until the sample is thoroughly charred. Cool then moisten the residue with 0.5-1 mL of sulfuric acid. Heat at a low temperature until white fumes are no longer evolved, and ignite at 700-800°C until the residue is completely incinerated. Cool the crucible in a desiccator, weigh accurately. Ignite at 700-800 °C again and cool until the weight is constant.

Calculation:

$$\text{Residue on ignition (\%)} = \frac{M_2 - M_1}{M_0} \times 100\%$$

In which,
M_0: Weight of sample before drying, g;
M_1: Weight of empty crucible after ignition at 700-800°C, g;
W_2: Weight of weighing bottle and sample after ignition at 700-800°C, g;

Test one sample and the result should be not more than 1.0%.

Related Substances and Purity

Reagents

Dipotassium hydrogen phosphate
Purified water
Acetonitrile

Chromatographic System

Equipment	Agilent 1200 HPLC
Column	Agilent Eclipse Plus C18 (250 mm × 4.6 mm, 5 μm)
Column Temperature	30°C
Injection volume	20 μL
Detector	UV at 254 nm
Flow rate	1.0 mL/min
Run time	30 min

Mobile Phase:

Time/min	**10 mM K_2HPO_4 (pH=8.0)/%**	**Acetonitrile/%**
0	80	20
10	80	20
25	40	60
30	40	60

Preparation of Solutions

Blank Solution: Prepare a mixture of acetonitrile and water (1:1)

Standard Solution: Accurately weigh about 25 mg of Reference Standard, and transfer to a 50 mL of volumetric flask. Dissolve and dilute with Blank Solution to volume, mix well.

Test Solution: Accurately weigh about 25 mg of sample and transfer to a 50 mL

volumetric flask. Dissolve and dilute with Blank Solution to volume, mix well. Prepare Test Solution 1 and Test Solution 2 in parallel.

Sensitive Solution: Pipette 1.0 mL of Test Solution to a 100 mL volumetric flask, dilute to volume with Blank Solution. Pipette 5.0 mL of this solution to a 100 mL volumetric flask, dilute to volume and mix well.

Procedure

Follow the injection sequence as defined in Table 3.2.S.2.3-3. Record the chromatograms.

Table 3.2.S.2.3-3 Injection sequence-Related Substances and Purity

Sequence	Frequency of Injection
Blank Solution	1
Sensitive Solution	1
System Suitability Solution/Test Solution 1	5
Test Solution 2	1
Standard Solution (for identification)	1

Calculation

Related Substances: $\text{Result} = \frac{r_u}{r_s} \times 100\%$

In which,

r_u: peak area response of each impurity from the Test Solution;

r_s: Sum of the peak area responses of all the peaks excluding solvents from the Test Solution.

Purity: $\text{Result} = \frac{r_u}{r_s} \times 100\%$

In which,

r_u: peak area response of principle peak from the Test Solution;

r_s: Sum of the peak area responses of all the peaks excluding solvents from the Test Solution.

System Suitability

The signal-to-noise ratio of principle peak from Sensitive Solution should be not less than 10; The RSD of principle peak areas from five replicate injections of system Suitability Solution should be not more than 2.0%; the Resolution, R, between principle peak and adjacent peak obtained with System Suitability Solution should be not less than

1.5 and the tailing factor of principle peak should be between 0.8 and 1.5.

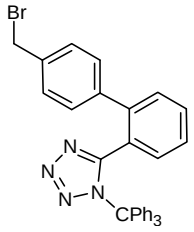
Acceptance Criteria

Any impurity should be not more than 0.2%; Total impurities should be not more than 1.0%; Purity should be not less than 99.0%.

4. Specification and analytical procedures of BBTT

Information on the specification of BBTT and the current qualified supplier is provided in Table 3.2.S.2.3-4

Table 3.2.S.2.3-4 Specification of BBTT

<table>
<tr><td colspan="2">Supplier:
Henan Huashang Pharmaceutical Co., Ltd.</td><td> 
</td><td>Mol. Formula:
$C_{33}H_{25}BrN_4$

Molecular Weight:
557.48</td></tr>
<tr><td colspan="2">Tests</td><td>Acceptance Criteria</td><td>Analytical Procedure</td></tr>
<tr><td colspan="2">Appearance</td><td>White or almost white crystalline powder</td><td>Visual examination</td></tr>
<tr><td colspan="2">Identification</td><td>The retention time of the major peak in the chromatogram of the Purity preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Purity.</td><td>HPLC</td></tr>
<tr><td colspan="2">Loss on drying</td><td>Not more than 1.0%</td><td>Drying</td></tr>
<tr><td rowspan="4">Related substances</td><td>Impurity RRT=1.1</td><td>Not more than 2.0%</td><td rowspan="4">HPLC</td></tr>
<tr><td>Impurity RRT=1.3</td><td>Not more than 2.0%</td></tr>
<tr><td>Any other impurity</td><td>Not more than 0.5%</td></tr>
<tr><td>Total impurities</td><td>Not more than 4.0%</td></tr>
<tr><td colspan="2">Purity</td><td>Not less than 96.0%</td><td>Ch.P.2010II Annex VD</td></tr>
</table>

BBTT is accepted based on a certificate of analysis (COA) from the supplier and on meeting internal quality standards following in-house tests.

A representative COA received from the current supplier of BBTT, as well as a COA based on in-house testing results provided in Fig. 3.2.S.2.3-5 and Fig. 3.2.S.2.3-6 respectively. An English translation of the test results is provided followed.

河南华商药业有限公司 Henan Huashang Pharmaceutical Co., Ltd.
No.168 West Chunshui Road, Zhecheng County, Shangqiu City, Henan Province, P.R. China
TEL: 086-370-7295678 FAX: 086-370-7296888 P.O.: 476200
http://www.huashangpharma.com

检 验 报 告 单

CERTIFICATE OF ANALYSIS

N-(三苯基甲基)-5-(4'-溴甲基联苯-2-基)四氮唑 CAS[124750-51-2]
N-(TRIPHENYLMETHYL)-5-(4'-BROMOMETHYLBIPHENYL-2-YL)TETRAZOLE

批 号 BATCH NO.	120204	生产日期 Manufacture date	Feb. 17, 2012
包 装 Packing	25Kg/Drum	保 质 期 Expiry date	Feb. 16, 2014
数 量 Quantity	750Kg	检验日期 Report date	Feb. 17, 2012

检测项目 Test Item	检测标准 Specification	检测结果 Results
外观 Appearance	白色或类白色结晶性粉末 White to off-white crystalline	符合 Qualified
鉴定方法 Identification	液相色谱保留时间同标准品 Retention time similar to standard	符合 Qualified
干燥失重 Loss on drying	Not more than 1.0%	0.16%
相关物质 Related substances		
N-(三苯基甲基)-5-[4'-甲基联苯-2-基]四氮唑 *N*-(Triphenylmethyl)-5-[4'-(methylbiphenyl)-2-yl]tetrazole	Not more than 2.0%	1.04%
N-(三苯基甲基)-5-[4' 4'-(二溴甲基联苯-2-基] 四氮唑 *N*-(Triphenylmeghyl)-5-[4'4'-(didromomethylbiphenyl)-2-yl] tetrazole	Not more than 2.0%	0.90%
分析含量 Purity (HPLC)	Not less than 96.0%	97.56%
结论： 符合企业标准 It complies with all the requirements of the Enterprise Standard.		

Reviser： Xiao Guo Inspector: Mengmeng Huangfu

3.2.S.2.3-5 A Representative COA of BBTT Provided by the Current Supplier

文件编号：TS-Y-A185 版本号：00
记录编号：TS-Y-A185a

宜昌长江药业有限公司
Yichang Changjiang Pharmaceutical CO.,LTD

N-(三苯基甲基)-5-(4'-溴甲基联苯-2-基)四氮唑 (Y744)检验报告书

Certificate of Analysis for n-(triphenylmethyl)-5-(4'-bromomethylbiphenyl-2-yl-)terazole

内部批号	Y744-120302	检验单号	Y$_3$-1203037
物料来源	河南华商药业有限公司	供应商批号	120204
送检时间	2012 年 03 月 27 日	送检部门	长江药业原料仓库(2)
报告时间	2012 年 06 月 01 日	检品数量	750kg
检验依据	N-(三苯基甲基)-5-(4'-溴甲基联苯-2-基)四氮唑质量标准（TS-Y-A185,00 版）		

检验项目	标准规定	检验结果
【外观】	为白色或类白色晶体粉末	为类白色粉末
【鉴别】	供试品与对照品主峰保留时间一致	符合规定
【干燥失重】	不得过 1.0%	0.13%
【有关物质】	三苯基四氮唑（RRT=1.1）不得过 2.0%	1.2%
	二溴代四氮唑（RRT=1.3）不得过 2.0%	0.96%
	其他最大单杂不得过 0.5%	小于定量限(0.05%)
	总杂不得过 4.0%	2.2%
【纯度】	不得少于 96.0%	97.8%

结论：本品检测结果符合 TS-Y-A185,00 版 规定。

批准人：2012.06.01 审核人：2012.6.01 打印人：2012.06.01

Fig. 3.2.S.2.3-6 A Representative COA of BBTT Provided by Changjiang Pharm

An English Translation of COA of BBTT Generated in Changjiang Pharm is showed in following page

Document No.: TS-Y-A185 Version: 00
Record No.: TS-Y-A185a Yichang Changjiang Pharmaceutical CO., LTD

Certificate of Analysis for
n-(triphenylmethyl)-5-(4'-bromomethylbiphenyl-2-yl-)terazole

Internal Batch No.	Y744-120302	**Test Sheet No.**	Y_2-1203037
Manufacturer	Henan Huashang Pharmaceutical Co., Ltd.	**Supplier Batch No.**	120204
Sampling date	27-MAR-2012	**Sampling Dept.**	API Warehouse (2)
Reporting date	01-JUN-2012	**Quantity**	750 kg
Standard	Quality Standard of BBTT (TS-Y-A185, version: 00)		

Test	**Acceptance criteria**	**Results**
Appearance	White or almost white crystalline powder	Almost white powder
Identification	The retention time of the major peak in the chromatogram of the Purity preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Purity.	Conforms
Loss on drying	Not more than 1.0%	0.13%
Related substances	Impurity RRT=1.1: Not more than 2.0%	1.2%
	Impurity RRT=1.3: Not more than 2.0%	0.96%
	Any other impurity : Not more than 0.5%	<0.05%
	Total impurities : Not more than 4.0%	2.2%
Purity	Not less than 96.0%	97.8%

Approved by: Haiyan Zhang 01-JUN-2012
Reviewed by: Yanhong Lan 01-JUN-2012
Printed by: Min Yao 01-JUN-201

Analytical Methods

Appearance

Visually examine the material and record the color and physical state. It should be white or almost white crystalline powder.

Identification

Proceed as directed in *Purity*. The retention time of the sample peak in the chromatogram corresponds to that obtained with the Standard Solution.

Loss on drying

Accurately weigh 1 g of the sample and place in a weighing bottle which has been dried at 105 °C to constant weight. Heat gradually to 105°C and dry at this temperature until constant weight is obtained.

Calculation:

$$X = \frac{W_1 + W_2 - W_3}{W_1} \times 100\%$$

In which,
X: Loss on drying, %;
W_1: Weight of sample before drying, g;
W_2: Weight of empty weighing bottle after drying at 105 °C, g;
W_3: Weight of weighing bottle and sample after drying at 105 °C, g;

Test two samples in parallel and use the arithmetical mean of the two results as the final result. If one test result fails to meet the acceptance criteria, the conclusion should be unqualified.

Acceptance criteria: The loss on drying should be not more than 1.0%.

Related Substances and Purity

Reagents
Purified water
Acetonitrile

Chromatographic System

Equipment	Agilent 1200 HPLC
Column	Agilent Eclipse XDB C18 (150 mm × 4.6 mm, 5 μm)
Column Temperature	30°C
Injection volume	20 μL
Detector	UV at 254 nm
Flow rate	1.0 mL/min
Run time	25 min

Mobile Phase: Prepare a filtered and degassed mixture of acetonitrile and water (80:20).

Preparation of Solutions

Blank Solution: acetonitrile

Test Solution: Accurately weigh about 10 mg of sample and transfer to a 50 mL volumetric flask. Dissolve and dilute with Blank Solution to volume, mix well.Prepare Test Solution 1 and Test Solution 2 in parallel.

System Suitability Solution: Test Solution 1

Standard Solution: Accurately weigh about 10 mg of Reference Standard and transfer to a 50 mL volumetric flask. Dissolve and dilute with Blank Solution to volume, mix well.

Sensitive Solution: Pipette 1.0 mL of Test Solution 1 to a 100 mL volumetric flask, dilute with Blank Solution and mix well. Pipette 5.0 mL of this solution to a 100 mL volumetric flask, dilute with Blank Solution to volume, and mix well.

Procedure

Follow the injection sequence as defined in Table 3.2.S.2.3-5. Record the chromatograms.

Table 3.2.S.2.3-5 Injection sequence-Related Substances and Purity

Sequence	Frequency of Injection
Blank Solution	1
Sensitive Solution	1
System Suitability Solution	5
Test Solution 2	1
Standard Solution (for identification)	1

Calculation

Related Substance:

$$\text{Result} = \frac{r_u}{r_s} \times 100\%$$

In which,

r_u: peak area response of each impurity from the Test Solution;

r_s: Sum of the peak area responses of all the peaks excluding solvents from the Test Solution.

Purity:

$$\text{Result} = \frac{r_u}{r_s} \times 100\%$$

In which,

r_u: peak area response of principle peak from the Test Solution;

r_s: Sum of the peak area responses of all the peaks excluding solvents from the Test Solution.

System Suitability

The RSD of principle peak areas from five replicate injections of System Suitability Solution should be not more than 2.0%; The signal-to-noise ratio of principle peak from Sensitive Solution should be not less than 10; the Resolution, R, between principle peak and adjacent peak from Test Solution should be not less than 1.5 and the tailing factor of principle peak should be between 0.8 and 1.5.

Acceptance Criteria

Impurity RRT=1.1 should be not more than 2.0%; Impurity RRT=1.3 should be not more than 2.0%; Any other impurity should be not more than 0.5%; Total impurities should be not more than 4.0%; Purity should be not less than 96.0%.

5. Statements of the starting Materials

Solvents of chloroform (class 2), methanol (class 2) and ethyl acetate (class 3) are used in manufacturing BDS, while solvents of dichloromethane (class 2), dimethylformamide (class 2), toluene (class 2) and ethanol (class 3) are used in manufacturing BBTT. No class 1 solvent is used in the BDS and BBTT production. All the residual solvents in BDS and BBTT are within the limits of ICH Q3C guideline and Ph. Eur. 2.4.24.

There are no TSE/BSE substances used or produced during the manufacturing of BDS

and BBTT.

The *Residual Solvents Statement* and *TSE/BSE Declaration* of BDS and BBTT are presented in the following pages.

河南华商药业有限公司 Henan Huashang Pharmaceutical Co., Ltd.
No.168 West Chunshui Road, Zhecheng County, Shangqiu City, Henan Province, P.R. China
TEL: 086-370-7295566 FAX: 086-370-7296888 P.O.: 476200
http://www.huashangpharma.com

RESIDUAL SOLVENTS STATEMENT

Product Name: **2-butyl-1,3-diazaspiro[4.4]non-1-en-4-one hydrochloride**

We, **Henan Huashang Pharmaceutical Co., Ltd.** (company name), hereby certify that the following solvents are used and controlled (as per ICH Q3C limits) for the above product supplying to **HEC Pharm. Co., Ltd.**

Solvent category (as per ICH Q3C)	Solvent name	Used in stage	Limits established
Class-1: Residual solvents:	NA	NA	NA
Class-2: Residual solvents:	Chloroform Methanol	Step1,2 Step 3	Not more than 60ppm Not more than 3000ppm
Class-3: Residual solvents:	Ethyl acetate	Step 3	Not more than 5000ppm
Other residual solvents (Not part of the above category):	NA	NA	NA

Note: If no solvent used, pl. mention as "NA".

COMPANY NAME: **Henan Huashang Pharmaceutical Co., Ltd.**

AUTHORIZED SIGNATORY:

NAME: **Xiao Guo**

DESIGNATION: **Quality Manager**

DATE: **2012-09-12**

2012.09.12

Fig. 3.2.S.2.3-7 Residual Solvents Statement of BDS

河南华商药业有限公司 Henan Huashang Pharmaceutical Co., Ltd.
No.168 West Chunshui Road, Zhecheng County, Shangqiu City, Henan Province, P.R. China
TEL: 086-370-7295566 FAX: 086-370-7296888 P.O.: 476200
http://www.huashangpharma.com

DECLARATION – TSE/BSE

Product Name: **2-butyl-1,3-diazaspiro[4.4]non-1-en-4-one hydrochloride**

We, **Henan Huashang Pharmaceutical Co., Ltd.** (company name), here by certify that the above product manufactured by us / supplying to **HEC Pharm. Co., Ltd.** conforms to the following points:

- No ingredients of animal origin.
- No material derived from or exposed to animals affected by, or under quarantine, for transmitting animal spongiform encephalopathy / bovine spongiform encephalopathy.
- Our manufacturing facility used having no animal (or) animal products (or) animal by-products (or) veterinary vaccines (or) animal pathogens.

COMPANY NAME: **Henan Huashang Pharmaceutical Co., Ltd.**

AUTHORIZED SIGNATORY:

NAME: **Xiao Guo**

DESIGNATION: **Quality Manager**

DATE: **2012-09-12**

2012.09.12

Fig. 3.2.S.2.3-8 TSE/BSE Declaration of BDS

河南华商药业有限公司 Henan Huashang Pharmaceutical Co., Ltd.
No.168 West Chunshui Road, Zhecheng County, Shangqiu City, Henan Province, P.R. China
TEL: 086-370-7295566 FAX: 086-370-7296888 P.O.: 476200
http://www.huashangpharma.com

RESIDUAL SOLVENTS STATEMENT

Product Name: N-(triphenylmethyl)-5-(4'-bromomethylbiphenyl-2-yl)tetrazole

We, Henan Huashang Pharmaceutical Co., Ltd. (company name), hereby certify that the following solvents are used and controlled (as per ICH Q3C limits) for the above product supplying to **HEC Pharm. Co., Ltd.**

Solvent category (as per ICH Q3C)	Solvent name	Used in stage	Limits established
Class-1: Residual solvents:	NA	NA	NA
Class-2: Residual solvents:	Dichloromethane DMF Toluene	Step1,2,3 Step1 Step3	Not more than 600ppm Not more than 880ppm Not more than 890ppm
Class-3: Residual solvents:	Ethanol	Step2,3	Not more than 5000ppm
Other residual solvents (Not part of the above category):	NA	NA	NA

Note: If no solvent used, pl. mention as "NA".

COMPANY NAME: **Henan Huashang Pharmaceutical Co., Ltd.**

AUTHORIZED SIGNATORY:

NAME: **Xiao Guo**

DESIGNATION: **Quality Manager**

DATE: **2012-09-12**

2012.09.12

Fig. 3.2.S.2.3-9 Residual Solvents Statement of BBTT

河南华商药业有限公司 Henan Huashang Pharmaceutical Co., Ltd.
No.168 West Chunshui Road, Zhecheng County, Shangqiu City, Henan Province, P.R. China
TEL: 086-370-7295566 FAX: 086-370-7296888 P.O.: 476200
http://www.huashangpharma.com

DECLARATION – TSE/BSE

Product Name: **N-(triphenylmethyl)-5-(4'-bromomethylbiphenyl-2-yl)tetrazole**

We, **Henan Huashang Pharmaceutical Co., Ltd.** (company name), here by certify that the above product manufactured by us / supplying to **HEC Pharm. Co., Ltd.** conforms to the following points:

- No ingredients of animal origin.
- No material derived from or exposed to animals affected by, or under quarantine, for transmitting animal spongiform encephalopathy / bovine spongiform encephalopathy.
- Our manufacturing facility used having no animal (or) animal products (or) animal by-products (or) veterinary vaccines (or) animal pathogens.

COMPANY NAME: **Henan Huashang Pharmaceutical Co., Ltd.**

AUTHORIZED SIGNATORY:

NAME: **Xiao Guo**

DESIGNATION: **Quality Manager**

DATE: **2012-09-12**

2012.09.12

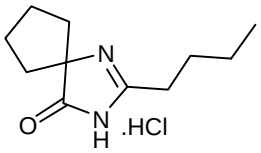
Fig. 3.2.S.2.3-10 TSE/BSE Declaration of BBTT

6. Structure Elucidation of Starting Materials

The chemical structures of starting materials BDS and BBTT for synthesis of irbesartan were confirmed.

6.1 Structure Elucidation of BDS

The chemical structure of BDS is shown as follows:



BDS

Batch Number of BDS: Y743-120301

The structure of BDS was confirmed by nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), Infrared spectrometry (IR), ultraviolet spectrometry (UV) and ion chromatography (IC).

6.1.1 Ultraviolet Spectroscopy

Instrument: Varian (Agilent) Carry 50 UV spectrophotometer

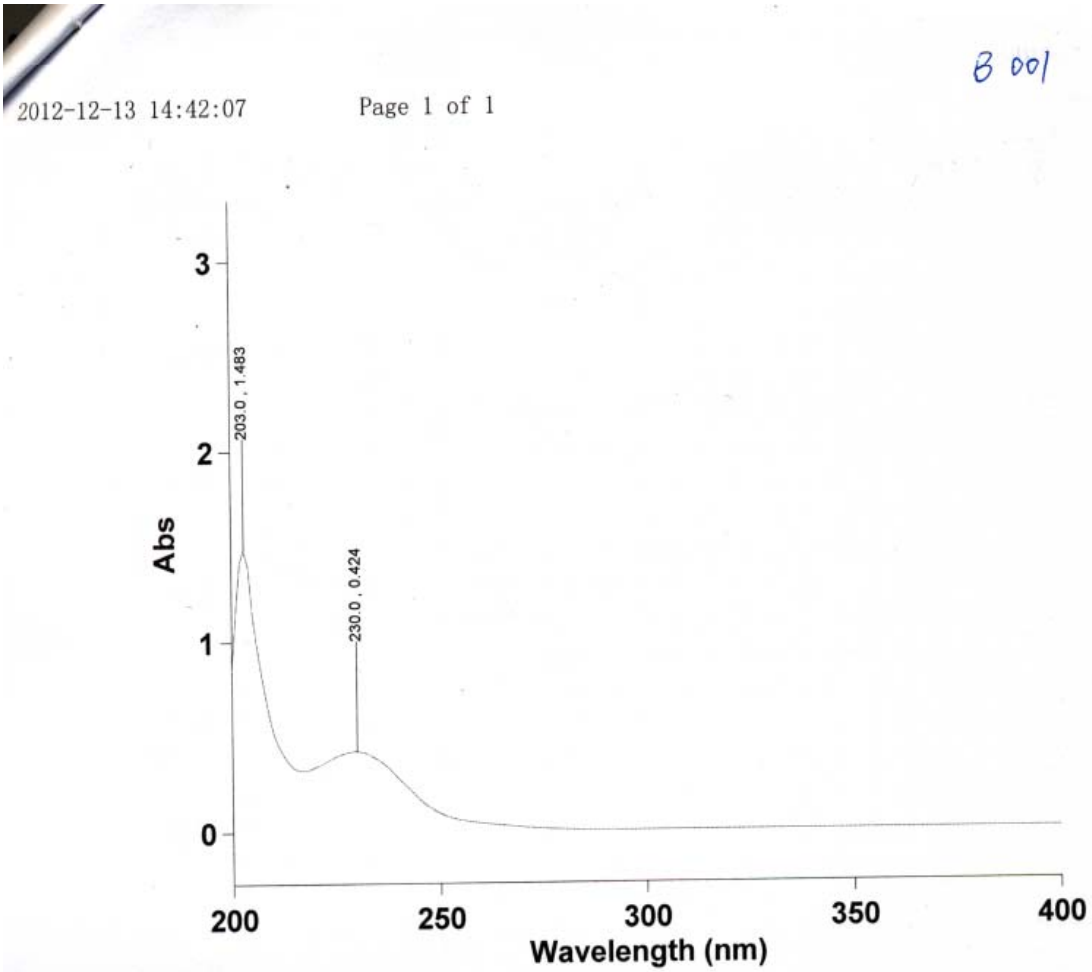
Solution: Separately dissolve samples of BDS in methanol, 0.1 mol/L sodium hydroxide-methanol solution, and 0.1 mol/L hydrochloric acid-methanol solution.

Results: The maximum absorption wavelengths of the solutions are listed in the following table, which indicate that the structure of the sample is consistent with that of BDS.

Table 3.2.S.2.3-6 UV Analysis Results of BDS

Solution	λ_{max2} (nm)	Absorbency
Methanol	203.0 230.0	1.483 0.424
0.1 mol/L sodium hydroxide - methanol solution	251.0	0.424
0.1 mol/L hydrochloric acid - methanol solution	203.0 230.0	0.445 1.504

Spectra: The spectra is presented below under the following titles:



Scan Analysis Report

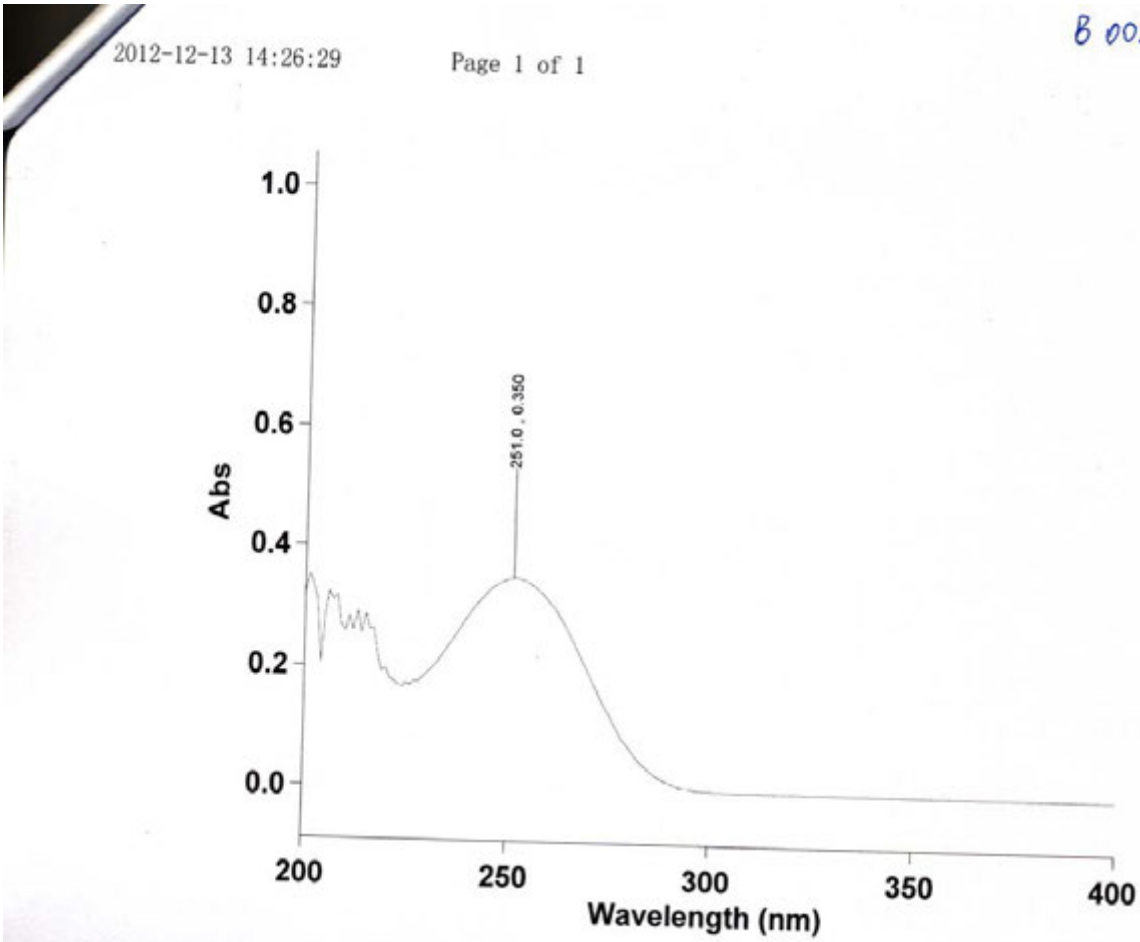
Report Time : 星期四 13 十二月 02:39:12 PM 2012
Method:
Batch: E:\JGQZ\20121213\Y743-120301-MeOH.DSW
Software version: 3.00(339)
Operator:

Sample Name: Y743-120301
Collection Time 2012-12-13 14:40:50

Peak Table
Peak Style Peaks
Peak Threshold 0.0100
Range 400.0nm to 200.0nm

Wavelength (nm)	Abs
230.0	0.424
203.0	1.483

Fig 3.2.S2.3-11 UV Spectrum of BDS in methanol



Scan Analysis Report

Report Time : 星期四 13 十二月 02:23:59 PM 2012
Method:
Batch: E:\JGQZ\20121213\Y743-120301-NaOH.DSW
Software version: 3.00(339)
Operator:

Sample Name: Y743-120301
Collection Time 2012-12-13 14:25:25

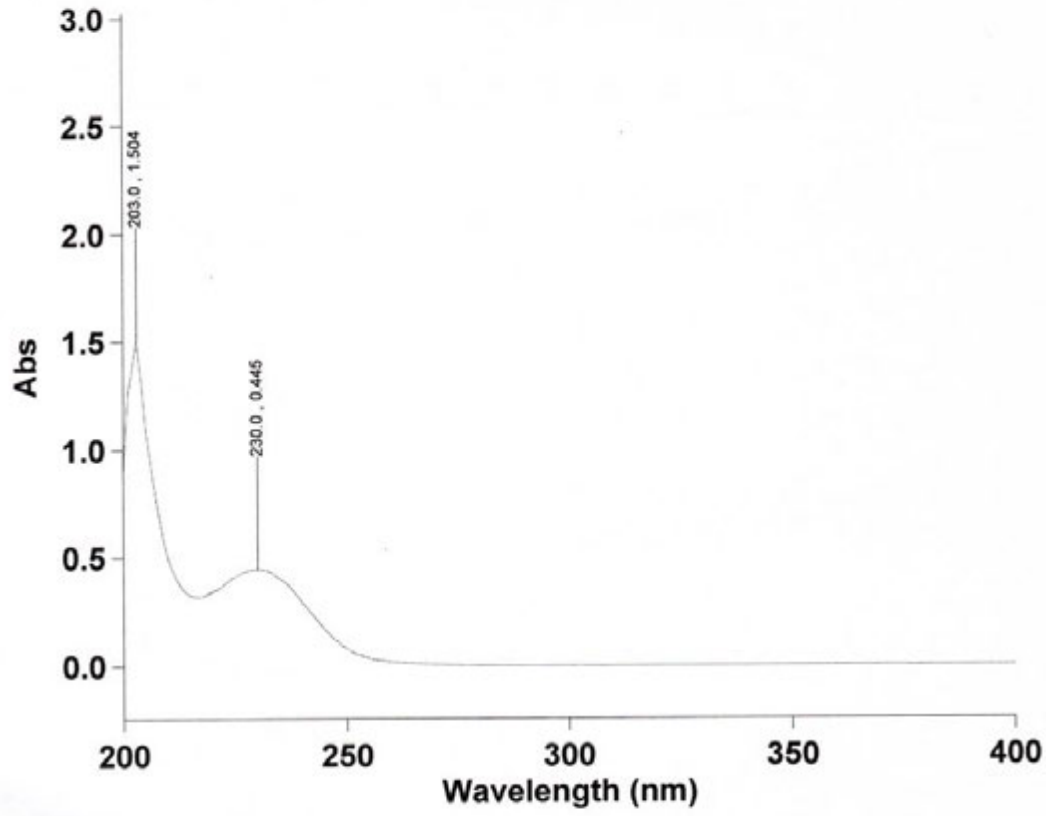
Peak Table
Peak Style Peaks
Peak Threshold 0.0100
Range 400.0nm to 200.0nm

Wavelength (nm)	Abs
251.0	0.350
215.1	0.287
213.0	0.291
211.0	0.283
206.0	0.323
201.1	0.353

Fig 3.2.S2.3-12 UV Spectrum of BDS in 0.1 mol/L sodium hydroxide-methanol solution

B003

2012-12-13 14:47:14 Page 1 of 1



Scan Analysis Report

Report Time : 星期四 13 十二月 02:45:09 PM 2012
Method:
Batch: E:\JGQZ\20121213\Y743-120301-HCL.DSW
Software version: 3.00(339)
Operator:

Sample Name: Y743-120301

Collection Time	2012-12-13 14:46:33
Peak Table	
Peak Style	Peaks
Peak Threshold	0.0100
Range	400.0nm to 200.0nm

Wavelength (nm)	Abs
230.0	0.445
203.0	1.504

Fig 3.2.S2.3-13 UV Spectrum of BDS in 0.1 mol/L hydrochloric acid-methanol

solution

6.1.2 Infrared Spectroscopy

Instrument: NICOLET IS10 Fourier Transform Infrared Spectrometer

Result: The main absorption peaks and other relevant spectral information in the spectra from the sample are listed in Table 3.2.S.2.3-7, which indicate the structure of the sample is consistent with that of BDS.

Table 3.2.S.2.3-7 The IR Spectra Data of BDS

Absorption Peak/cm^{-1}	**Vibration type**	**Function group**	**Strength**
2962～2633	ν_{C-H}	-CH_2-, -CH_3	Middle
1778	$\nu_{C=O}$	Lactam	Strong
1643	δ_{N-H}	-CO-NH-	Middle
1518	$\nu_{C=N}$	Nitrogen heterocyclic ring	Strong

Spectra: The spectrogram is presented below under the following titles.

Fig. 3.2.S.2.3-14 IR spectrum of BDS

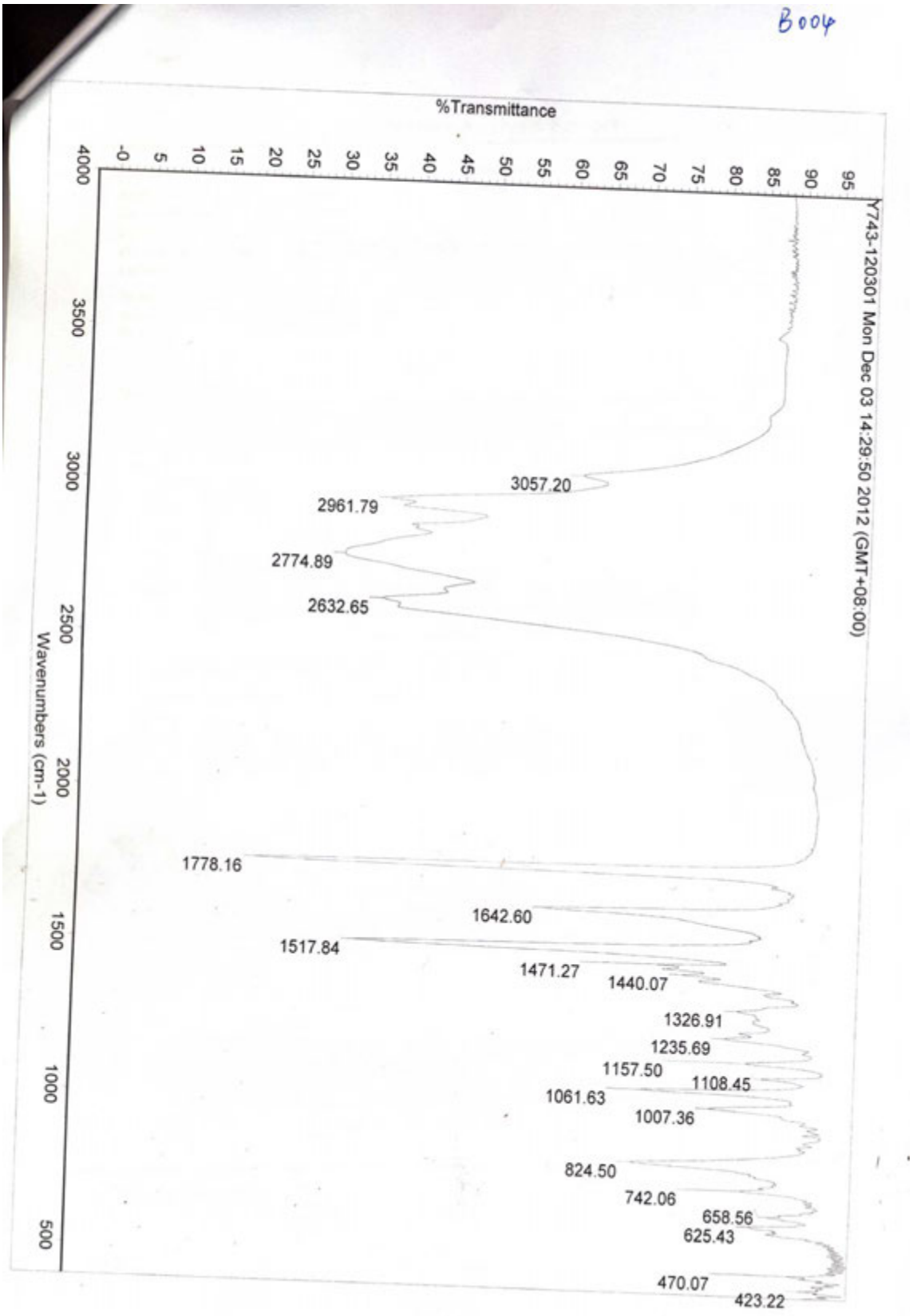


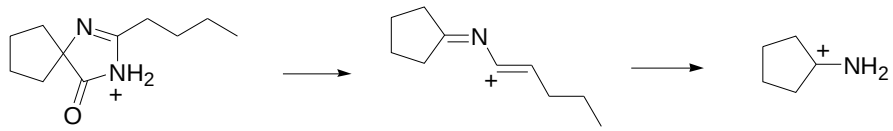
Fig. 3.2.S.2.3-14 IR spectrum of BDS

6.1.3 Mass Spectrometry

Instrument: Agilent 1260 HPLC, Agilent 6530 Q-TOF

Experimental condition: ESI Positive, AUTOMS (2) Mode

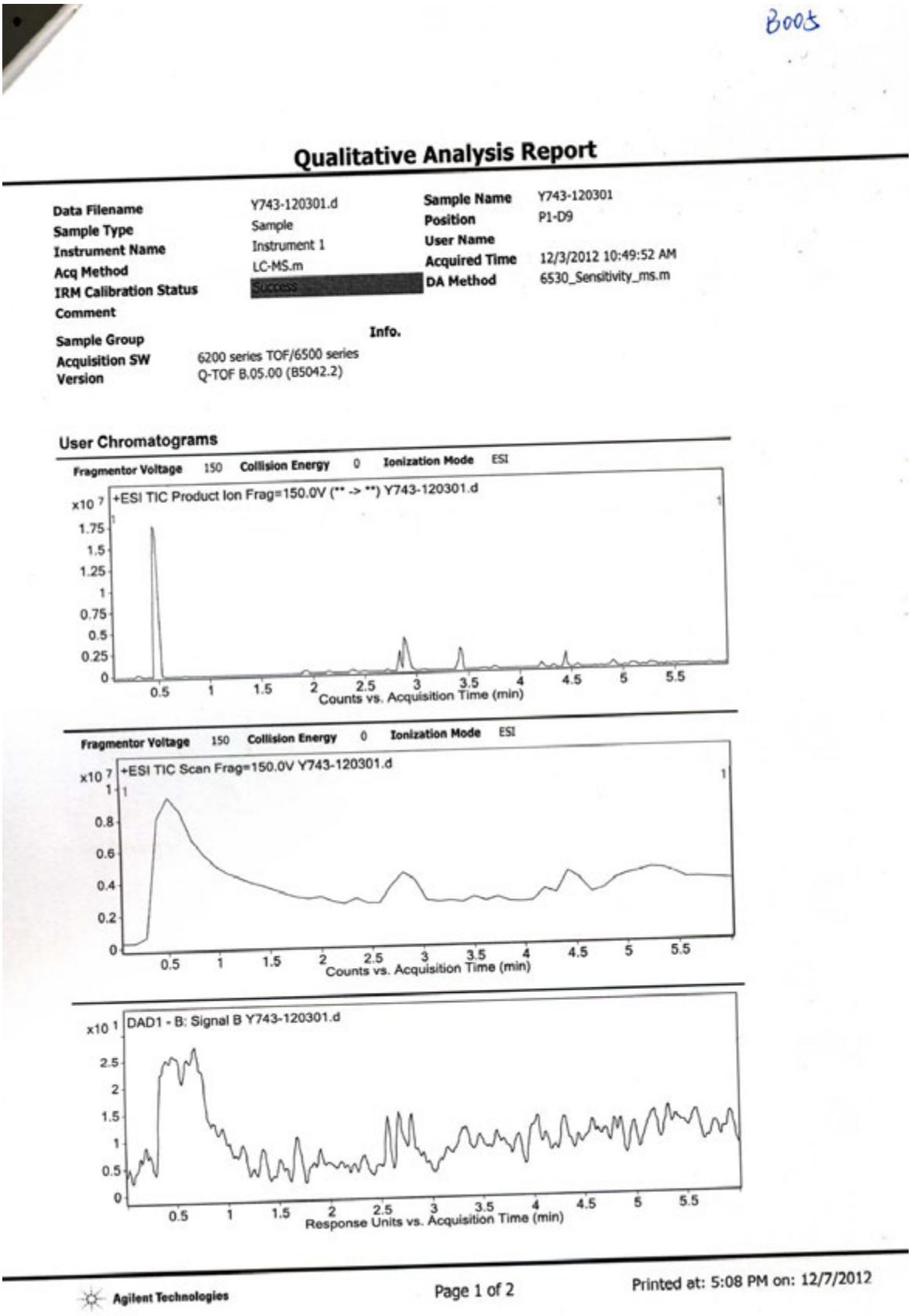
Analysis: The $[M + H]^+$ ion at m/z 195 obtained with ESI full scan mode suggests a molecular weight 194u which corresponds to the that of BDS without hydrochloride. With the AUTOMS (2) mode, the ion at m/z 195 produces 2 significant ions at m/z 150 and 84, respectively. The molecular fragments corresponding to these 2 ions and the MS fragmentation mechanism are shown below:



m/z 195.1492 m/z 150.1277 m/z 84.0808

Spectra: The spectra obtained with BDS is presented below with the following titles:

Fig 3.2.S.2.3-15 MS Spectrum of BDS



B003

Qualitative Analysis Report

Data Filename	Y743-120301.d	Sample Name	Y743-120301
Sample Type	Sample	Position	P1-D9
Instrument Name	Instrument 1	User Name	
Acq Method	LC-MS.m	Acquired Time	12/3/2012 10:49:52 AM
IRM Calibration Status	Success	DA Method	6530_Sensitivity_ms.m
Comment			

Info.

Sample Group	
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.00 (B5042.2)

User Chromatograms

Fragmentor Voltage 150 Collision Energy 0 Ionization Mode ESI

Fragmentor Voltage 150 Collision Energy 0 Ionization Mode ESI

Agilent Technologies Page 1 of 2 Printed at: 5:08 PM on: 12/7/2012

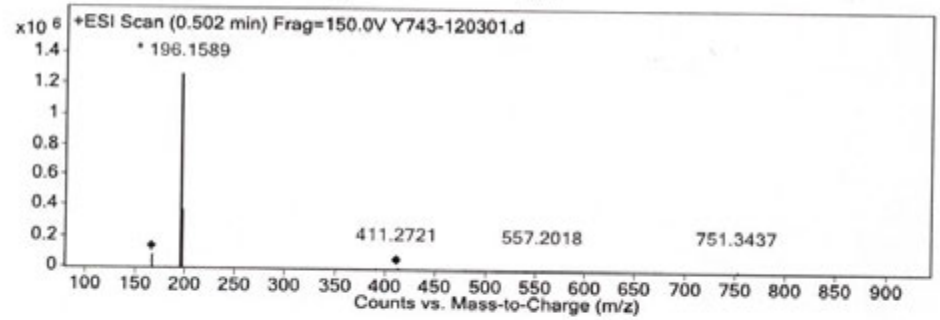
Fig 3.2.S.2.3-15 MS Spectrum of BDS

B006

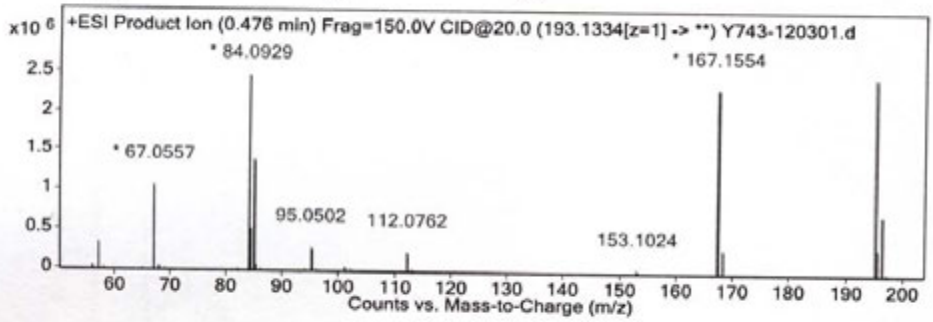
Qualitative Analysis Report

User Spectra

Fragmentor Voltage: 150 | Collision Energy: 0 | Ionization Mode: ESI



Fragmentor Voltage: 150 | Collision Energy: 20 | Ionization Mode: ESI



--- End Of Report ---

Fig 3.2.S.2.3-15 MS Spectrum of BDS (continued)

6.1.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Instrument: Superconducting Fourier Transform Nuclear magnetic Resonance Spectrometry (Bruker AVANCE AV 400)

Solvent: DMSO

Results: ^{1}H-NMR spectra shows 17 proton signals, which stand for 3 protons from methyl group and 14 protons from methylene group. ^{13}C-NMR spectra shows 9 carbon signals which stand for 11 carbons, including 1 carbon from methyl group, 7 carbons from methylene group and 3 quaternary carbons. And the HSQC, HMBC(^{1}H→^{13}C) and ^{1}H-^{1}H COSY spectrum further confirm the structure. The spectral data are listed in the below table.

Table 3.2.S.2.3-8 HSQC, HMBC(^{1}H→^{13}C) and 1H-1H COSY Spectral Data

No.	HSQC		HMBC(^{1}H→^{13}C)	^{1}H-^{1}H COSY
	δ_H	δ_C		
1	2.00 (m, 2H)	36.5	C-2, 3, 4, 5, 6	H-2
2	1.90 (m, 1H), 1.83 (m, 1H)	24.9	C-1, 3, 4, 5	—
3	1.90 (m, 1H), 1.83 (m, 1H)	36.5	C-1, 2, 4, 5	—
4	2.00 (m, 2H)	24.9	C-1, 2, 3, 5, 6	H-3
5	—	72.0	—	—
6	—	179.5	—	—
7	—	173.2	—	—
8	2.78 (t, *J* = 7.68, 2H)	27.3	C-7, 9, 10	H-9
9	1.73 (quint, *J* = 7.6, 2H)	26.6	C-7, 8, 10, 11	H-8, 10
10	1.34 (sext, *J* = 7.4, 2H)	21.4	C-9, 11	H-9, 11
11	0.90 (t, *J*=7.3 Hz, 3H)	13.3	C-9, 10	H-10

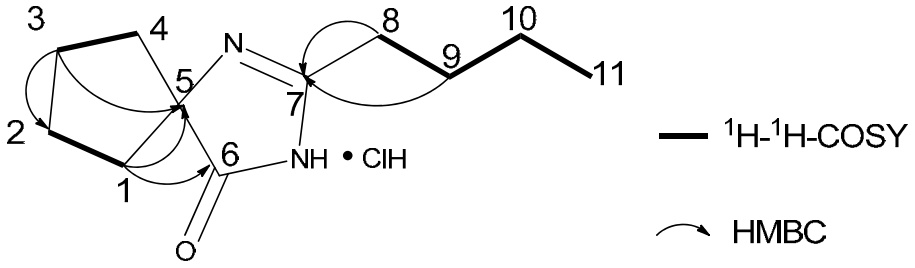


Fig 3.2.S.2.3-16 Main HMBC and ^{1}H-^{1}H COSY Correlations

Spectra: The spectrograms are presented below under the following titles.

Fig 3.2.S.2.3-17 ^{1}H-NMR spectrum of BDS
Fig 3.2.S.2.3-18 ^{13}C-NMR spectrum of BDS
Fig 3.2.S.2.3-19 HSQC spectrum of BDS
Fig 3.2.S.2.3-20 HMBC spectrum of BDS
Fig 3.2.S.2.3-21 ^{1}H-^{1}H COSY spectrum of BDS

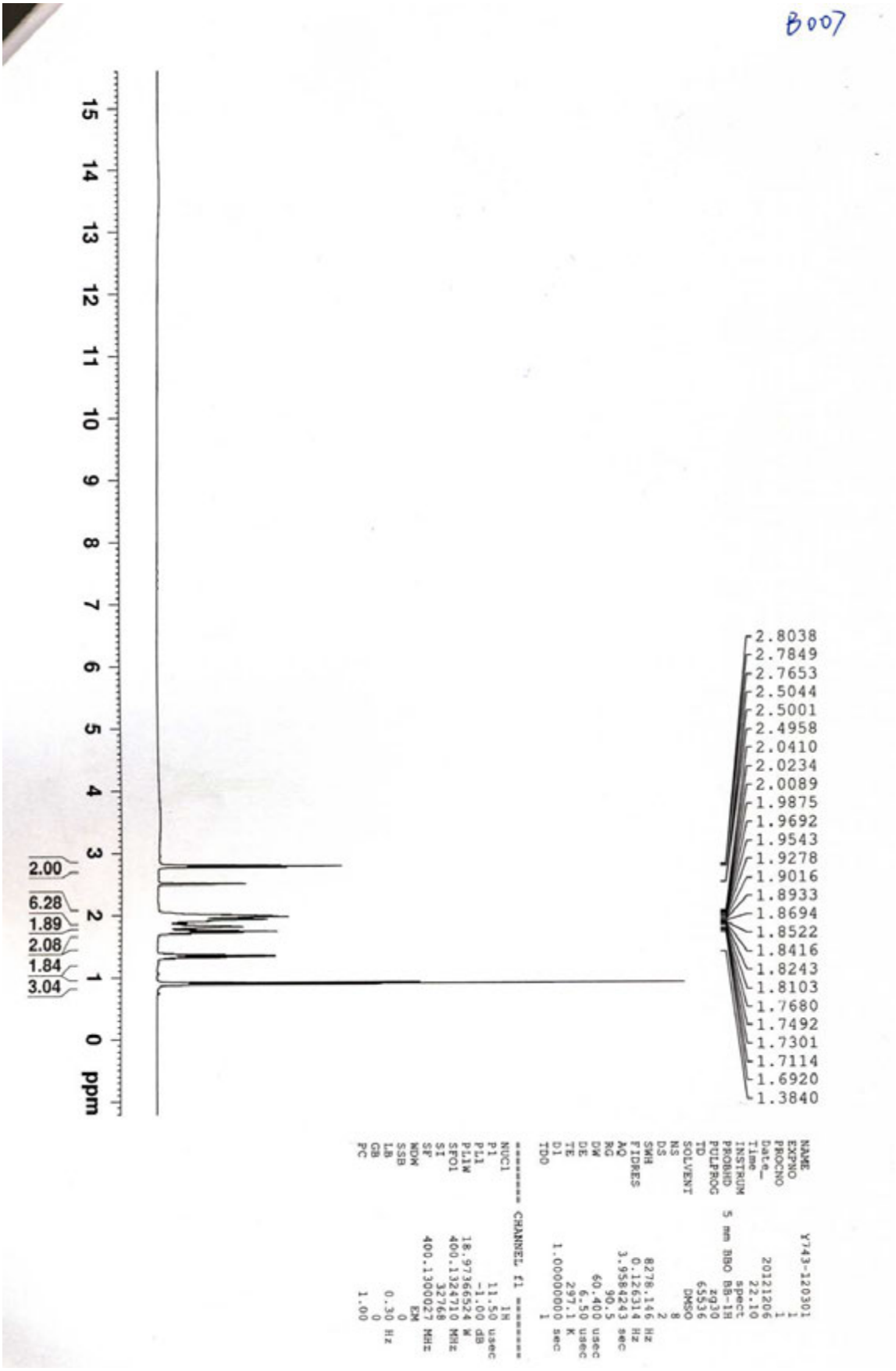


Fig 3.2.S.2.3-17 ^{1}H-NMR spectrum of BDS

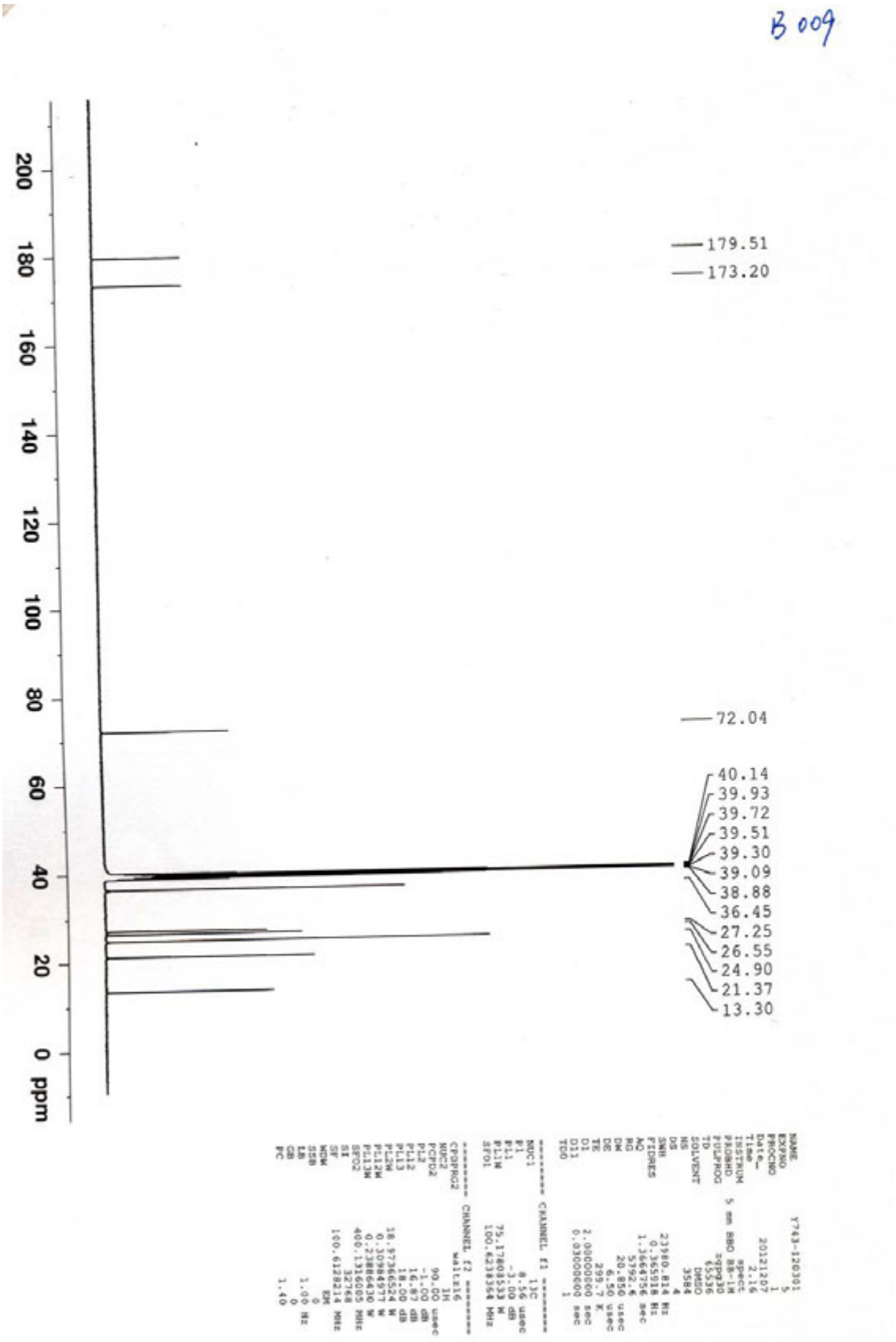


Fig 3.2.S.2.3-18 ^{13}C-NMR spectrum of BDS

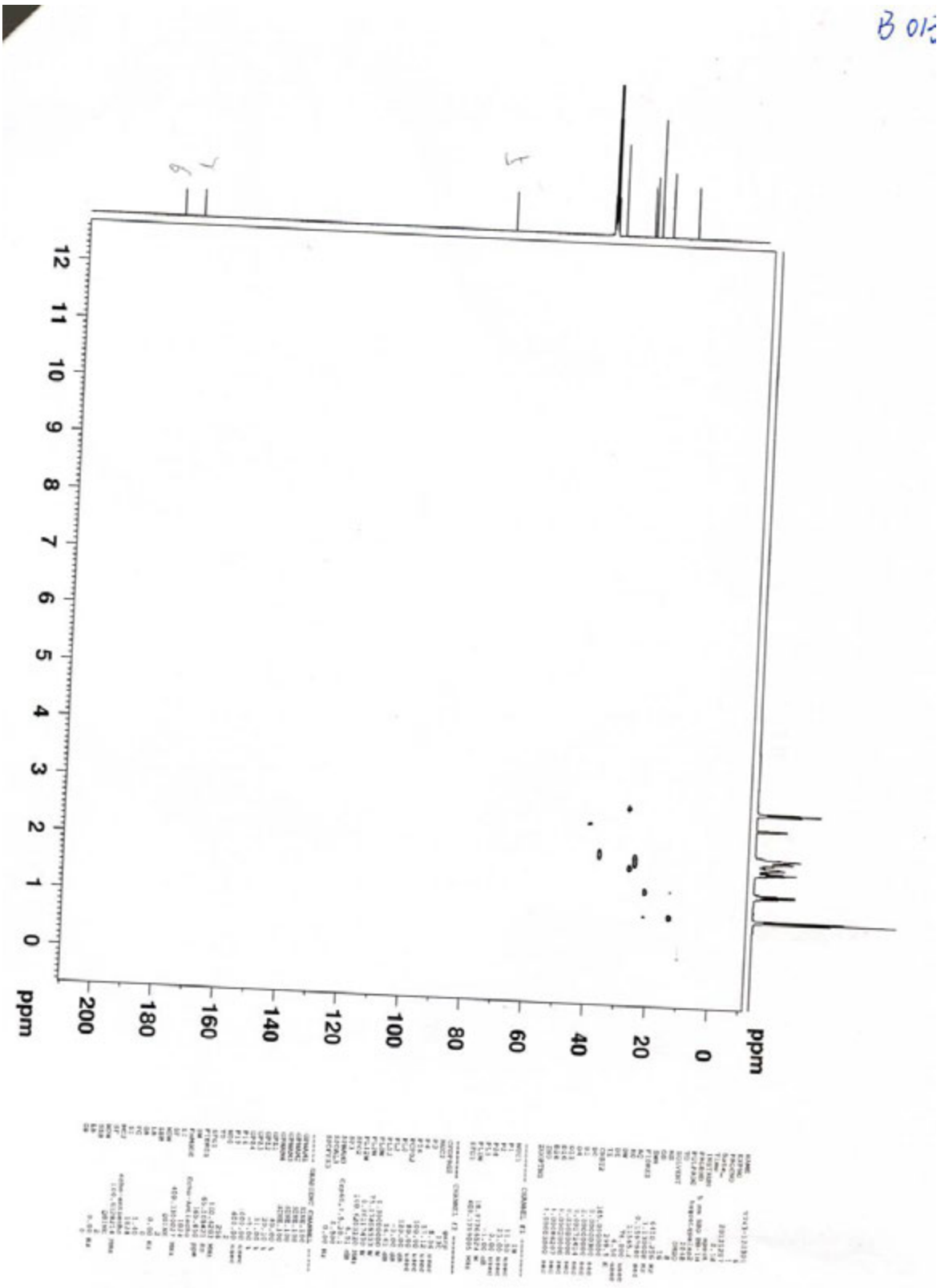


Fig 3.2.S.2.3-19 HSQC spectrum of BDS

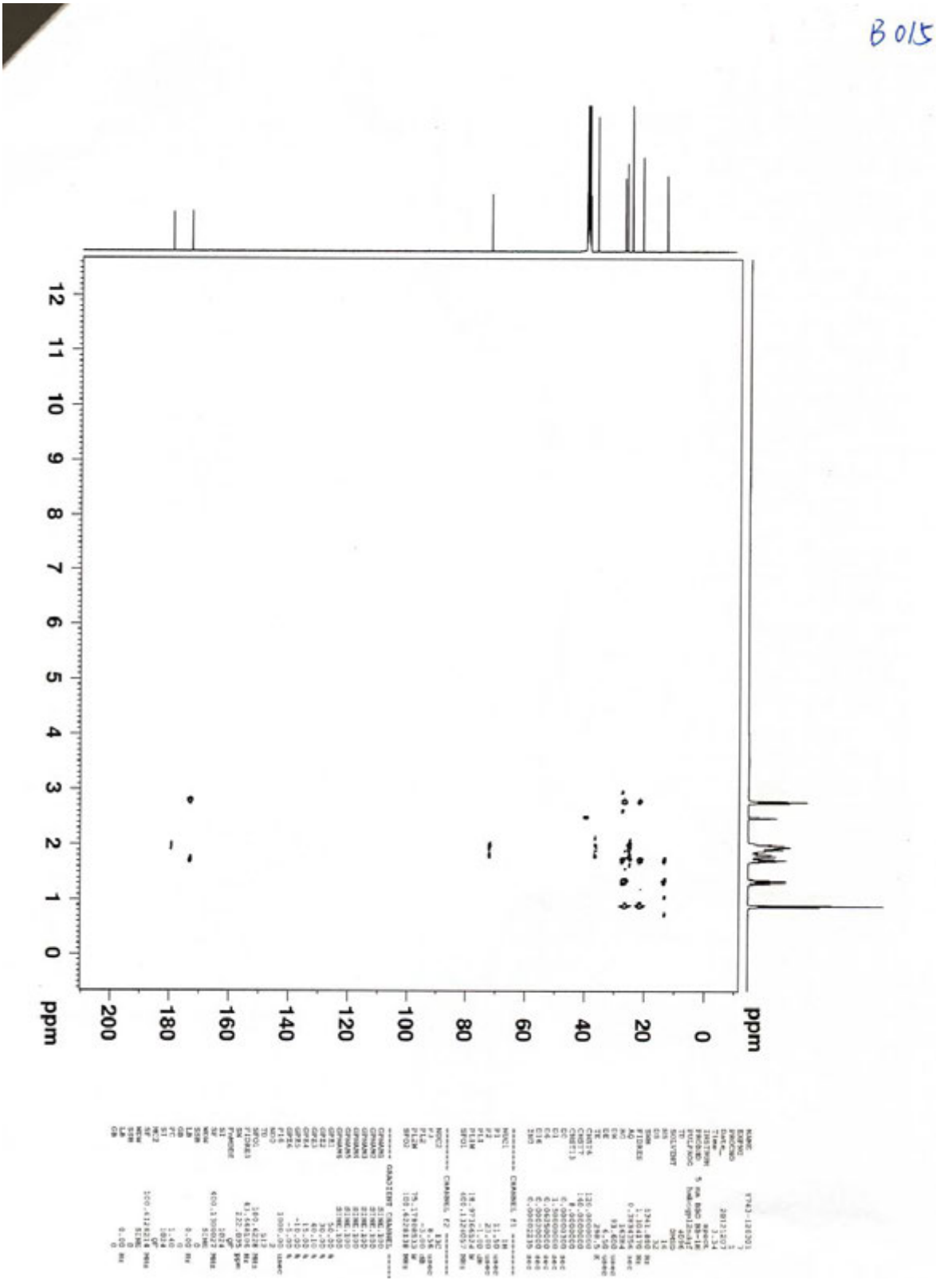


Fig 3.2.S.2.3-20 HMBC spectrum of BDS

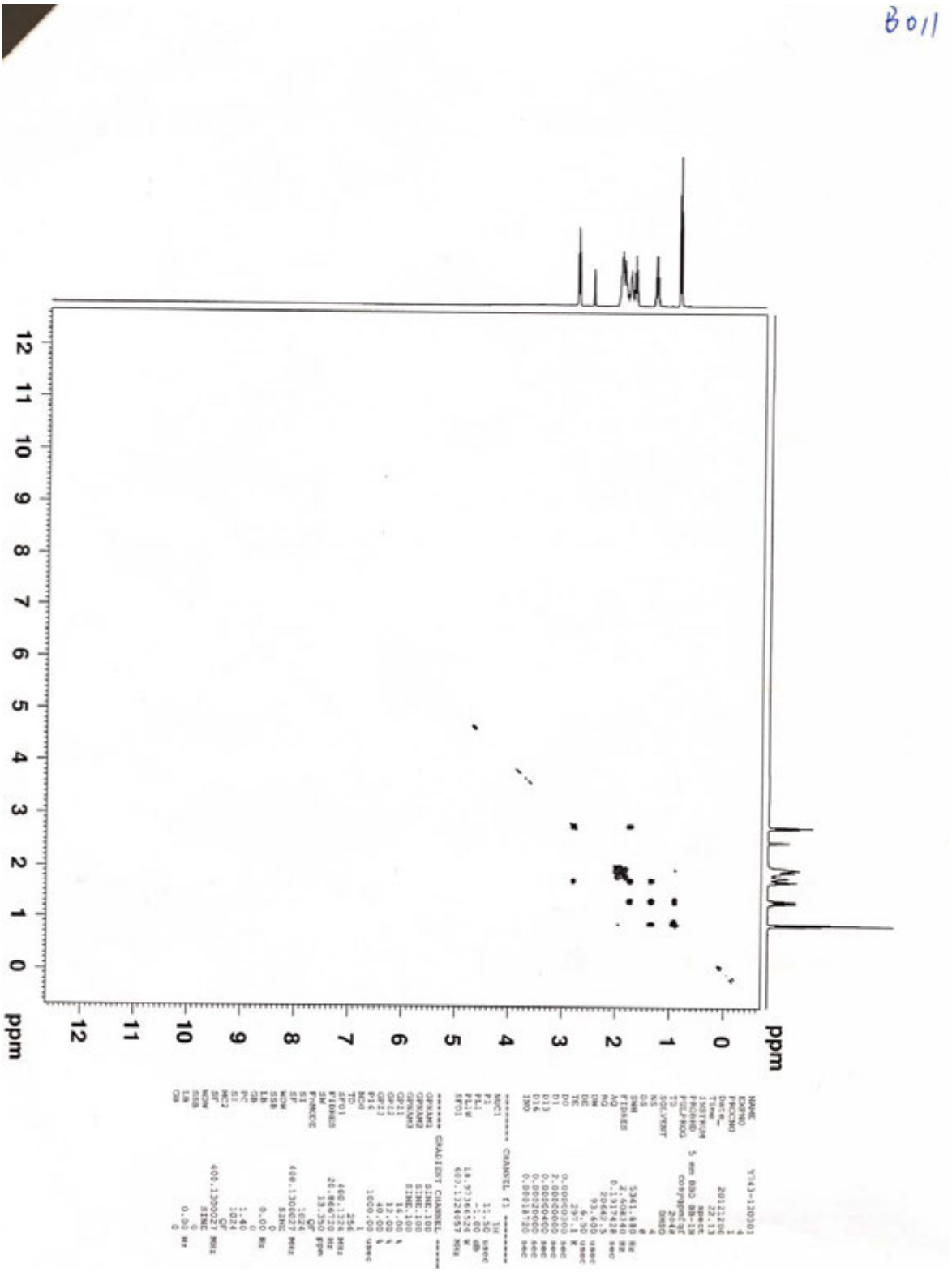
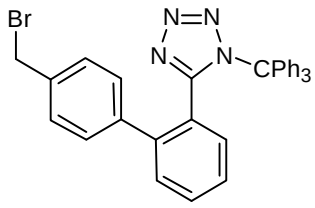


Fig 3.2.S.2.3-21 ^{1}H-^{1}H COSY spectrum of BDS

6.2 Structure Elucidation of BBTT

The chemical structure BBTT is shown as follows:



BBTT

Batch Number of BBTT: Y744-120301

The structure of BDS was confirmed by nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), Infrared spectrometry (IR), ultraviolet spectrometry (UV).

6.2.1 Ultraviolet Spectroscopy

Instrument: Varian (Agilent) Carry 50 UV spectrophotometer

Solution: Separately dissolve samples of BBTT in methanol, 0.1 mol/L sodium hydroxide-methanol solution, and 0.1 mol/L hydrochloric acid-methanol solution.

Results: The maximum absorption wavelengths of the solutions are listed in the following table, which indicate that the structure of the sample is consistent with that of BBTT.

Table 3.2.S.2.3-9 UV Analysis Results of BBTT

Solution	λ_{max2} (nm)	Absorbency
Methanol	205.0	2.475
0.1 mol/L sodium hydroxide - methanol solution	206.0 211.9 218.1	0.654 0.668 1.224
0.1 mol/L hydrochloric acid - methanol solution	204.1 255.9	2.716 0.462

Spectra: The spectra is presented below under the following titles:

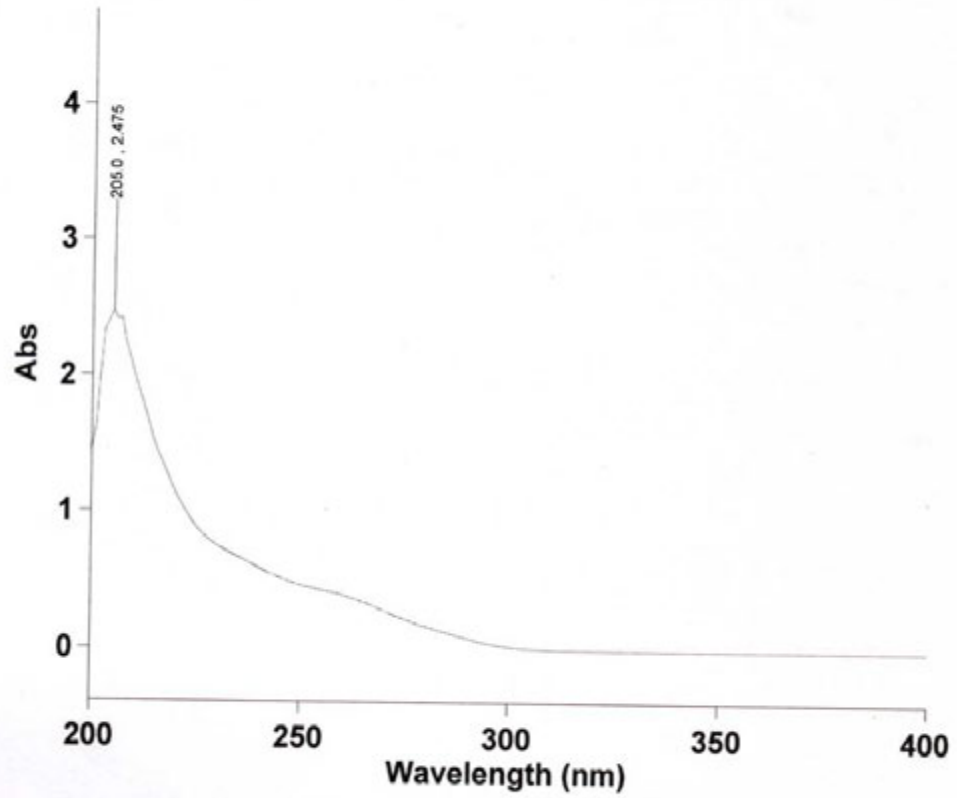
Fig 3.2.S2.3-22 UV Spectrum of BBTT in methanol

Fig 3.2.S2.3-23 UV Spectrum of BBTT in 0.1 mol/L sodium hydroxide-methanol solution

Fig 3.2.S2.3-24 UV Spectrum of BBTT in 0.1 mol/L hydrochloric acid-methanol solution

2012-12-13 14:35:06 Page 1 of 1

C 001



Scan Analysis Report

Report Time : 星期四 13 十二月 02:33:06 PM 2012
Method:
Batch: E:\JGQZ\20121213\Y744-120301-MeOH.DSW
Software version: 3.00(339)
Operator:

Sample Name: Y744-120301

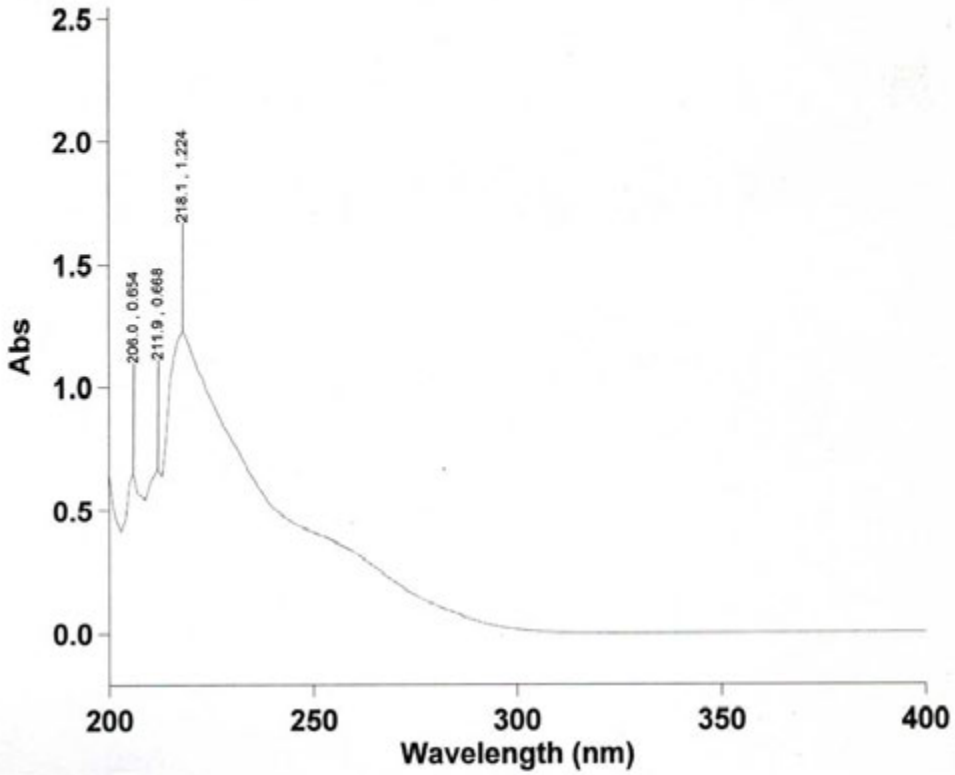
Collection Time	2012-12-13 14:34:27
Peak Table	
Peak Style	Peaks
Peak Threshold	0.0100
Range	400.0nm to 200.0nm

Wavelength (nm)	Abs
205.0	2.475

Fig 3.2.S2.3-22 UV Spectrum of BBTT in methanol

C002

2012-12-13 14:13:07 Page 1 of 1



Scan Analysis Report

Report Time : 星期四 13 十二月 02:11:24 PM 2012
Method:
Batch: E:\JGQZ\20121213\Y744-120301-NaOH.DSW
Software version: 3.00(339)
Operator:

Sample Name: Y744-120301

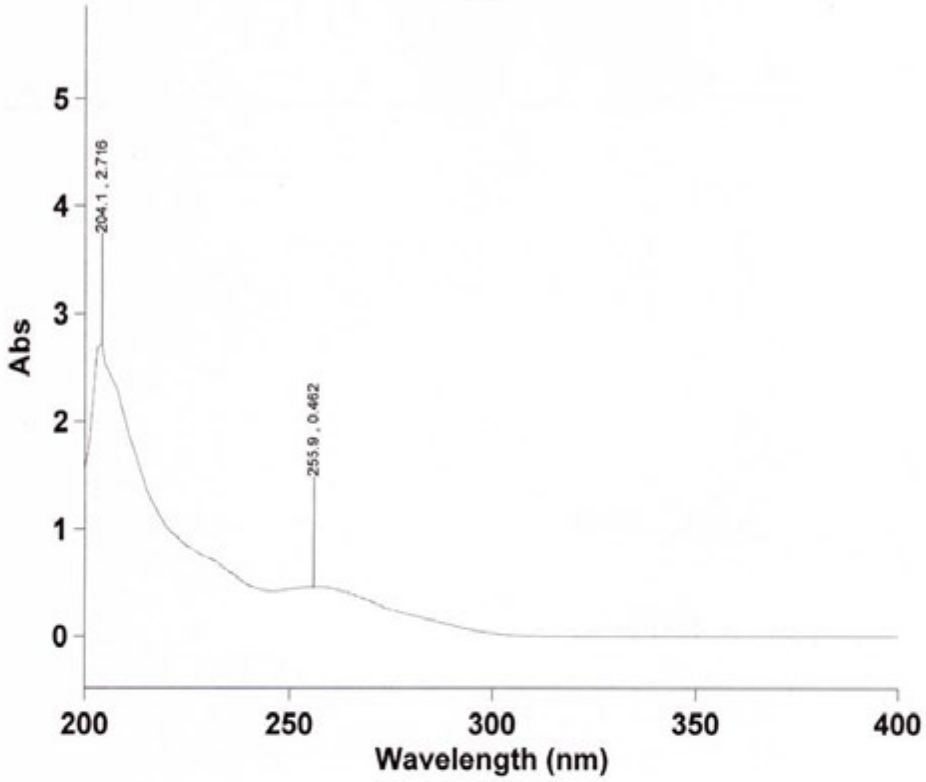
Collection Time	2012-12-13 14:11:34
Peak Table	
Peak Style	Peaks
Peak Threshold	0.0100
Range	400.0nm to 200.0nm

Wavelength (nm)	Abs
218.1	1.224
211.9	0.668
206.0	0.654

Fig 3.2.S2.3-23 UV Spectrum of BBTT in 0.1 mol/L sodium hydroxide-methanol solution

C 003

2012-12-13 14:53:01 Page 1 of 1



Scan Analysis Report

Report Time : 星期四 13 十二月 02:51:06 PM 2012
Method:
Batch: E:\JGQZ\20121213\Y744-120301-HCL.DSW
Software version: 3.00(339)
Operator:

Sample Name: Y744-120301

Collection Time	2012-12-13 14:52:18
Peak Table	
Peak Style	Peaks
Peak Threshold	0.0100
Range	400.0nm to 200.0nm

Wavelength (nm)	Abs
255.9	0.462
204.1	2.716

Fig 3.2.S2.3-24 UV Spectrum of BBTT in 0.1 mol/L hydrochloric acid-methanol solution

6.2.2 Infrared Spectroscopy

Instrument: NICOLET IS10 Fourier Transform Infrared Spectrometer

Result: The main absorption peaks and other relevant spectral information in the spectra from the sample are listed in Table 3.2.S.2.3-10, which indicate the structure of the sample is consistent with that of BBTT.

Table 3.2.S.2.3-10The IR Spectra Data of BBTT

Absorption Peak/cm^{-1}	**Vibration type**	**Function group**	**Strength**
3045, 3028	$\nu_{=C-H}$	Aromatic ring	Week
1606~1406	$\nu_{C=C,}$ δ_{C-H}	Aromatic ring, -CH_2-	Middle ~ Strong
1026, 1006, 881, 842	δ_{C-H}	1, 2 - substituted and 1, 4 - substituted aromatic	Middle
767~609	δ_{C-H}	Monosubstituted aromatic ring	Strong

Spectra: The spectrogram is presented below under the following titles.

Fig. 3.2.S.2.3-25 IR spectrum of BBTT

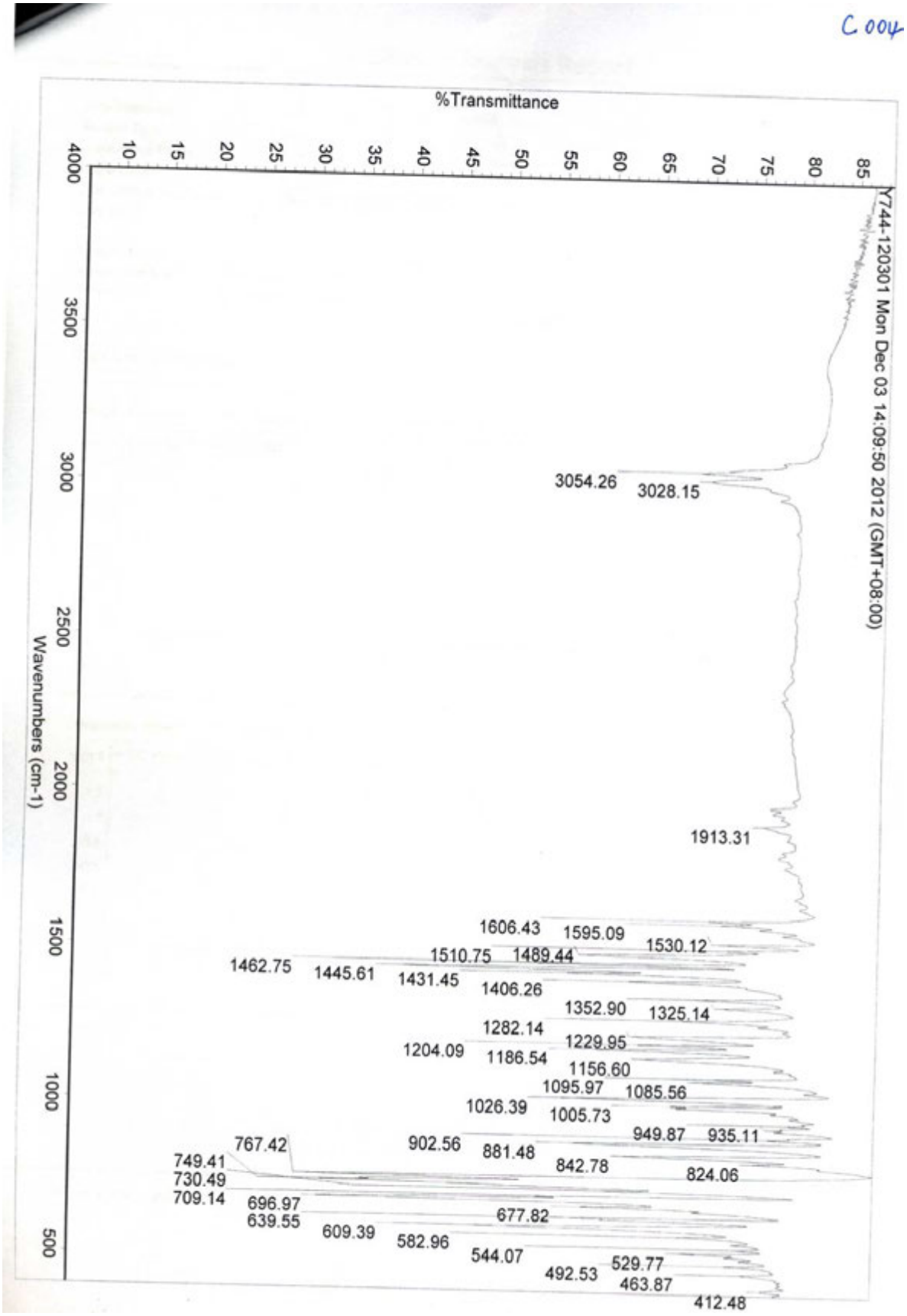


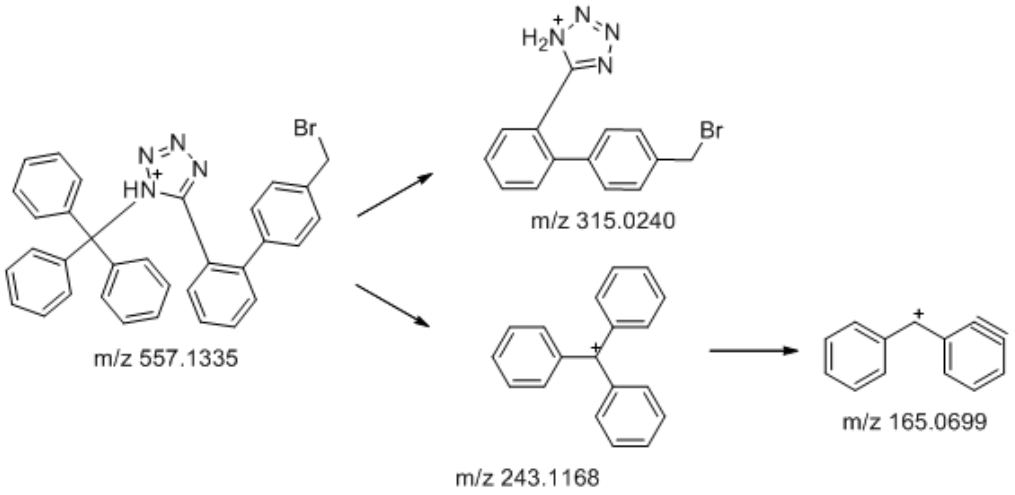
Fig. 3.2.S.2.3-25 IR spectrum of BBTT

6.2.3 Mass Spectrometry

Instrument: Agilent 1260 HPLC, Agilent 6530 Q-TOF

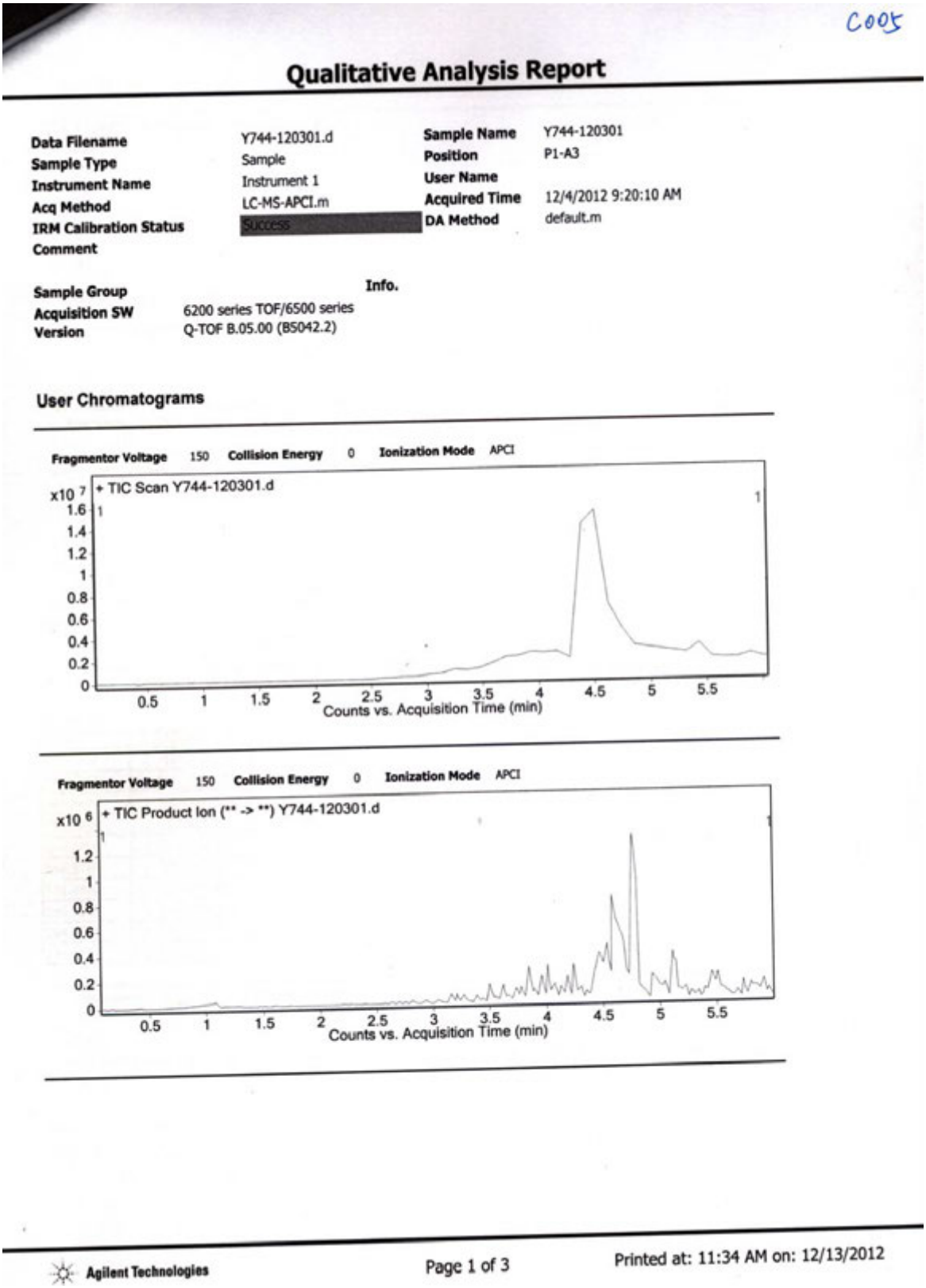
Experimental condition: APCI Positive, AUTOMS (2) Mode

Analysis: BBTT includes triphenyl group which is unstable under APCI and can easily decompose into two ions at 315 and 243. With the AUTOMS (2) mode, the ion at 243 decompose into another ion at 165. The molecular fragments and the MS fragmentation mechanism are shown below:



Spectra: The spectra obtained with BBTT is presented below with the following titles:

Fig 3.2.S.2.3-26 MS Spectrum of BBTT



C005

Qualitative Analysis Report

Data Filename	Y744-120301.d	Sample Name	Y744-120301
Sample Type	Sample	Position	P1-A3
Instrument Name	Instrument 1	User Name	
Acq Method	LC-MS-APCI.m	Acquired Time	12/4/2012 9:20:10 AM
IRM Calibration Status	Success	DA Method	default.m
Comment			

Sample Group	Info.
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.00 (B5042.2)

User Chromatograms

Fragmentor Voltage 150 Collision Energy 0 Ionization Mode APCI

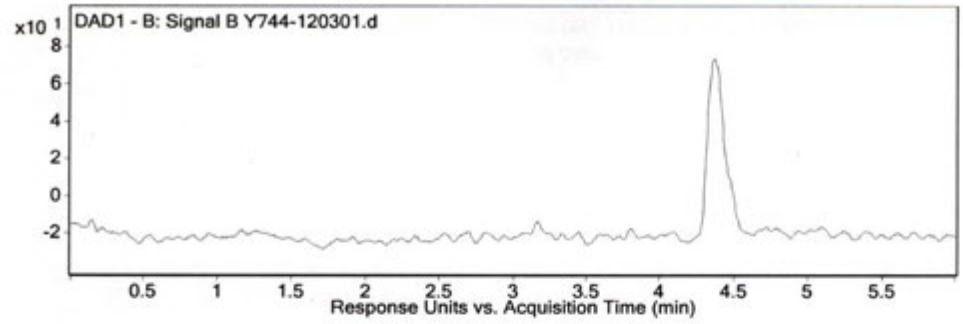
Fragmentor Voltage 150 Collision Energy 0 Ionization Mode APCI

Agilent Technologies Page 1 of 3 Printed at: 11:34 AM on: 12/13/2012

Fig 3.2.S.2.3-26 MS Spectrum of BBTT

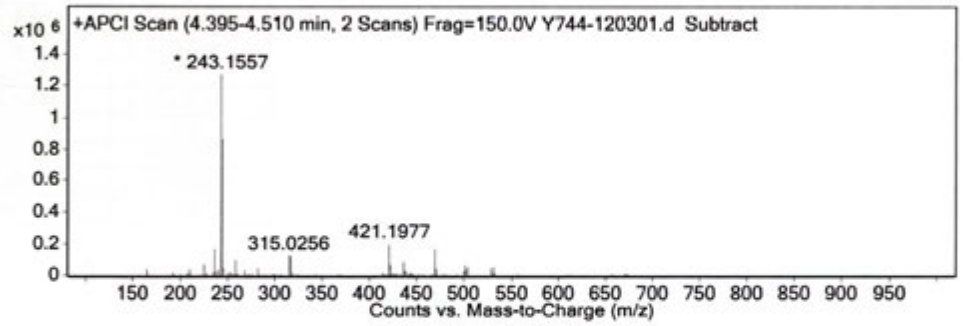
C006

Qualitative Analysis Report



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	APCI



Peak List

m/z	z	Abund
243.1557	2	1272452
243.2593		1223508.88
243.2975		628867.5
243.3219	2	350529.13
243.3459	2	320594.56
243.3766	2	300782.25
243.4478		357537.84
244.1346	2	1261090.13
244.2674	1	511170.75
245.125	2	864338.19

Fragmentor Voltage	Collision Energy	Ionization Mode
150	20	APCI

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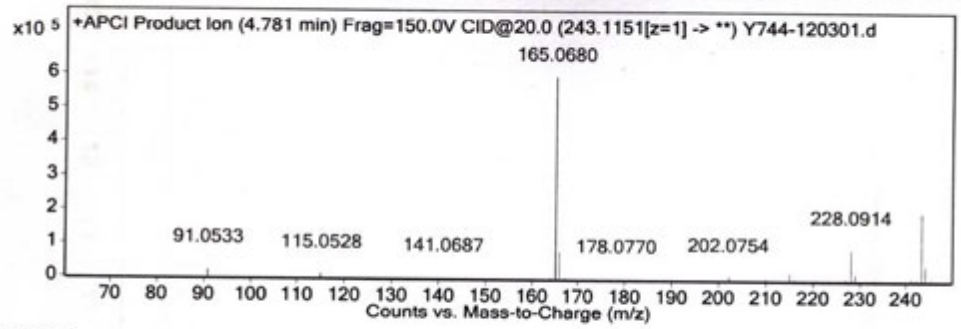
Fig 3.2.S.2.3-26 MS Spectrum of BBTT (continued)

C007

Qualitative Analysis Report



Peak List

m/z	z	Abund
91.0533	1	22872.69
115.0528	1	9082.44
165.068	1	597087.06
166.0713	1	78669.7
215.0838	1	23211.72
228.0914	1	90696.49
229.0948	1	17612.32
241.0982		7601.94
243.1147	1	200580.38
244.1183	1	41933.25

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Fig 3.2.S.2.3-26 MS Spectrum of BBTT (continued)

6.2.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Instrument: Superconducting Fourier Transform Nuclear magnetic Resonance Spectrometry (Bruker AVANCE AV 400)

Solvent: $CDCl_3$

Results: ^{1}H-NMR spectra shows 25 proton signals and ^{13}C-NMR spectra shows 17 carbon signals which stand for 33 carbons. And the HSQC, HMBC(^{1}H→^{13}C) and ^{1}H-^{1}H COSY spectrum further confirm the structure. The spectral data are listed in the below table.

Table 3.2.S.2.3-11 HSQC, HMBC(^{1}H→^{13}C) and 1H-1H COSY Spectral Data

No.	HSQC		HMBC(^{1}H→^{13}C)	^{1}H-^{1}H COSY
	δ_H	δ_C		
1	4.42 (2H, s)	33.4	C-2, 3	—
2	—	36.4	—	—
3, 3′	7.15 (2H, m)	28.7	C-1, 2, 4, 5	—
4, 4′	7.14 (2H, m)	29.8	C-2, 3, 5	—
5	—	41.5	—	—
6	—	41.7	—	—
7	—	26.5	—	—
8	8.02 (1H, m)	30.4	C-5, 10, 12	H-9
9	7.51 (1H, m)	27.9	C-6, 7, 8	H-8, 10
10	7.52 (1H, m)	30.1	C-6, 7, 8	H-11
11	7.42 (1H, m)	30.8	C-5, 7, 9	H-10
12	—	64.0	—	—
13	—	3.2	—	—
14a, 14b, 14c	—	41.4	—	—
15a, 15a′, 15b 15b′, 15c, 15c′	6.95 (6H, d, *J*=7.44Hz)	30.5	C-13, 17	H-16
16a, 16a′, 16b 16b′, 16c, 16c′	7.30 (6H, t, *J*=7.92Hz)	27.8	C-14, 15	H-15
17a, 17b, 17c	7.37 (3H, d, *J*=7.24Hz)	28.4	C-14, 15, 16	—

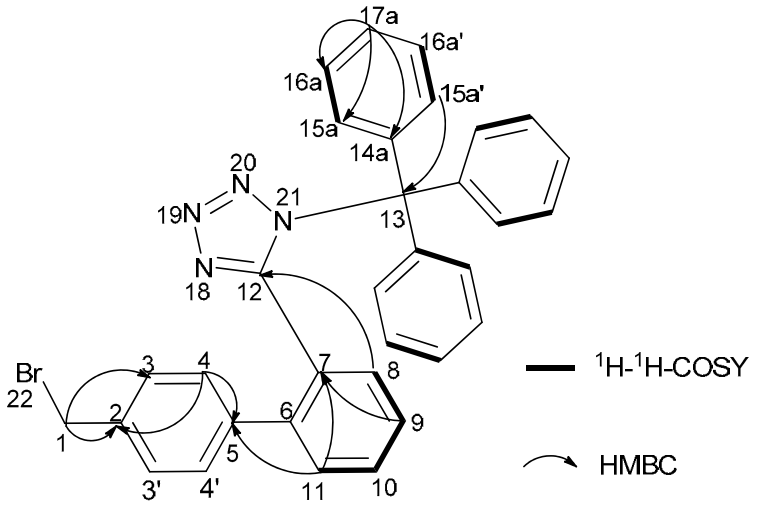

Fig 3.2.S.2.3-26Main HMBC and ^{1}H-^{1}H COSY Correlations

Spectra: The spectrograms are presented below under the following titles.

Fig 3.2.S.2.3-27 ^{1}H-NMR spectrum of BBTT
Fig 3.2.S.2.3-28 ^{13}C-NMR spectrum of BBTT
Fig 3.2.S.2.3-29 HSQC spectrum of BBTT
Fig 3.2.S.2.3-30 HMBC spectrum of BBTT
Fig 3.2.S.2.3-31^{1}H-^{1}H COSY spectrum of BBTT

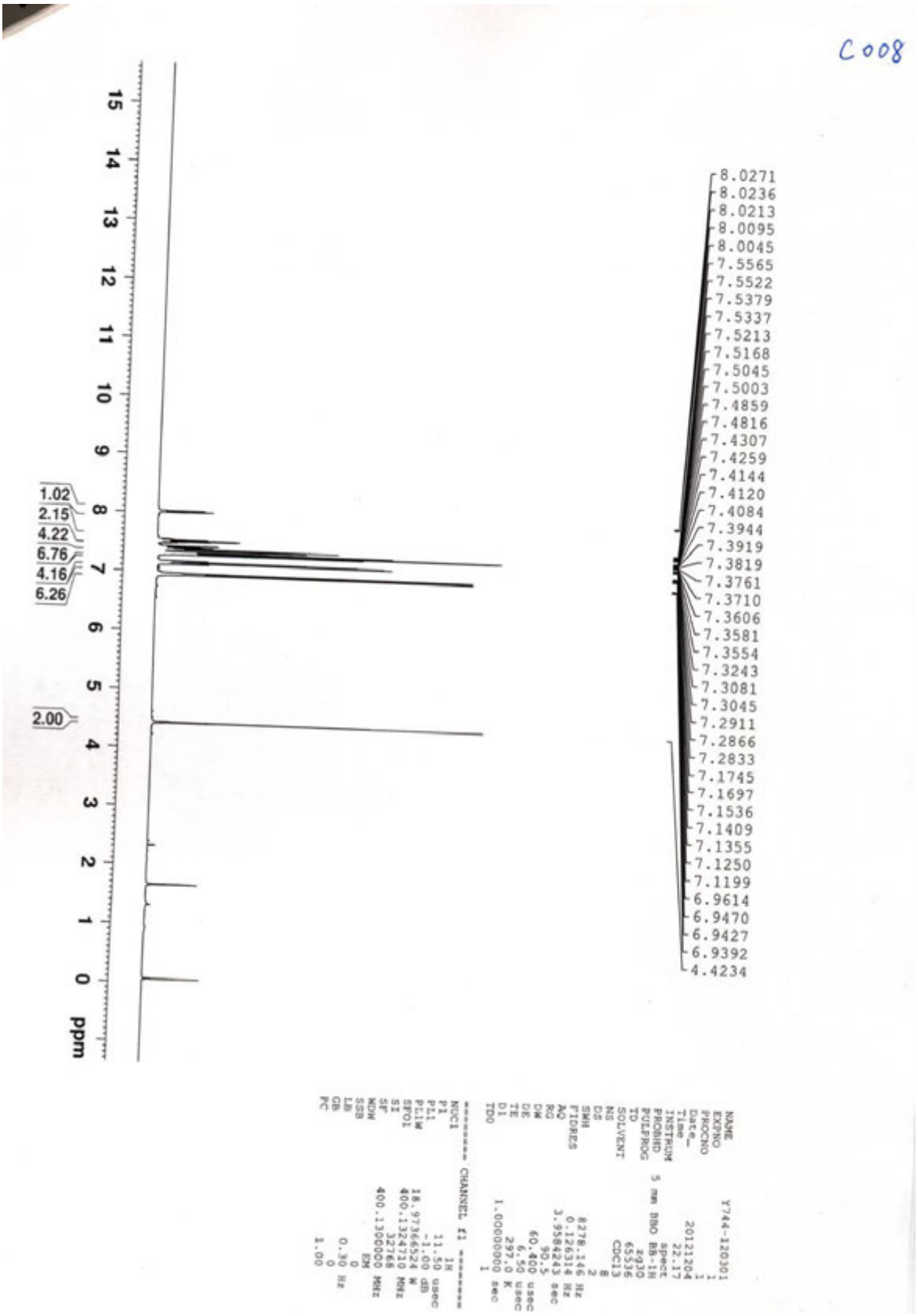

Fig 3.2.S.2.3-27 ^{1}H-NMR spectrum of BBTT

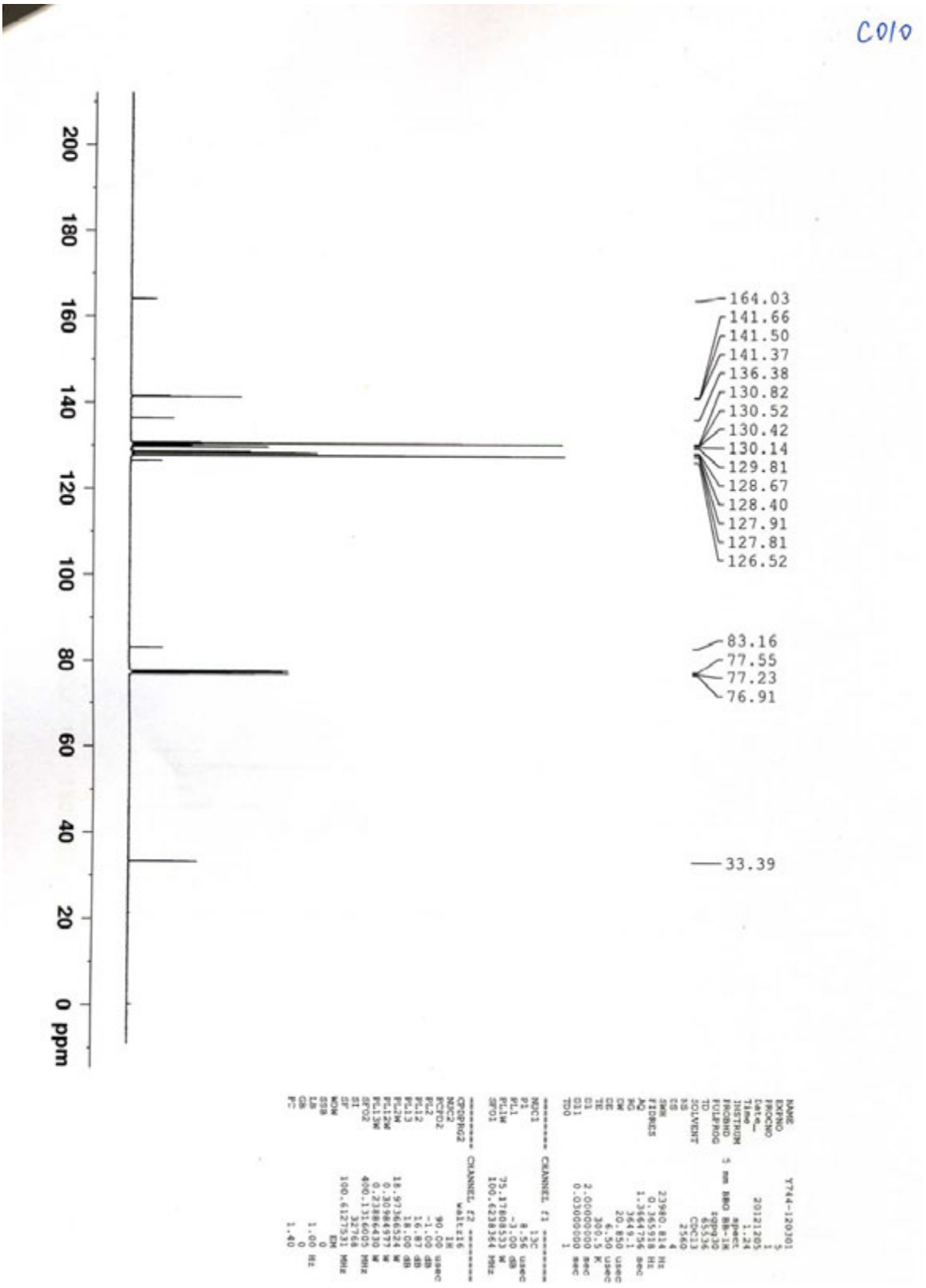

Fig 3.2.S.2.3-28 ^{13}C-NMR spectrum of BBTT

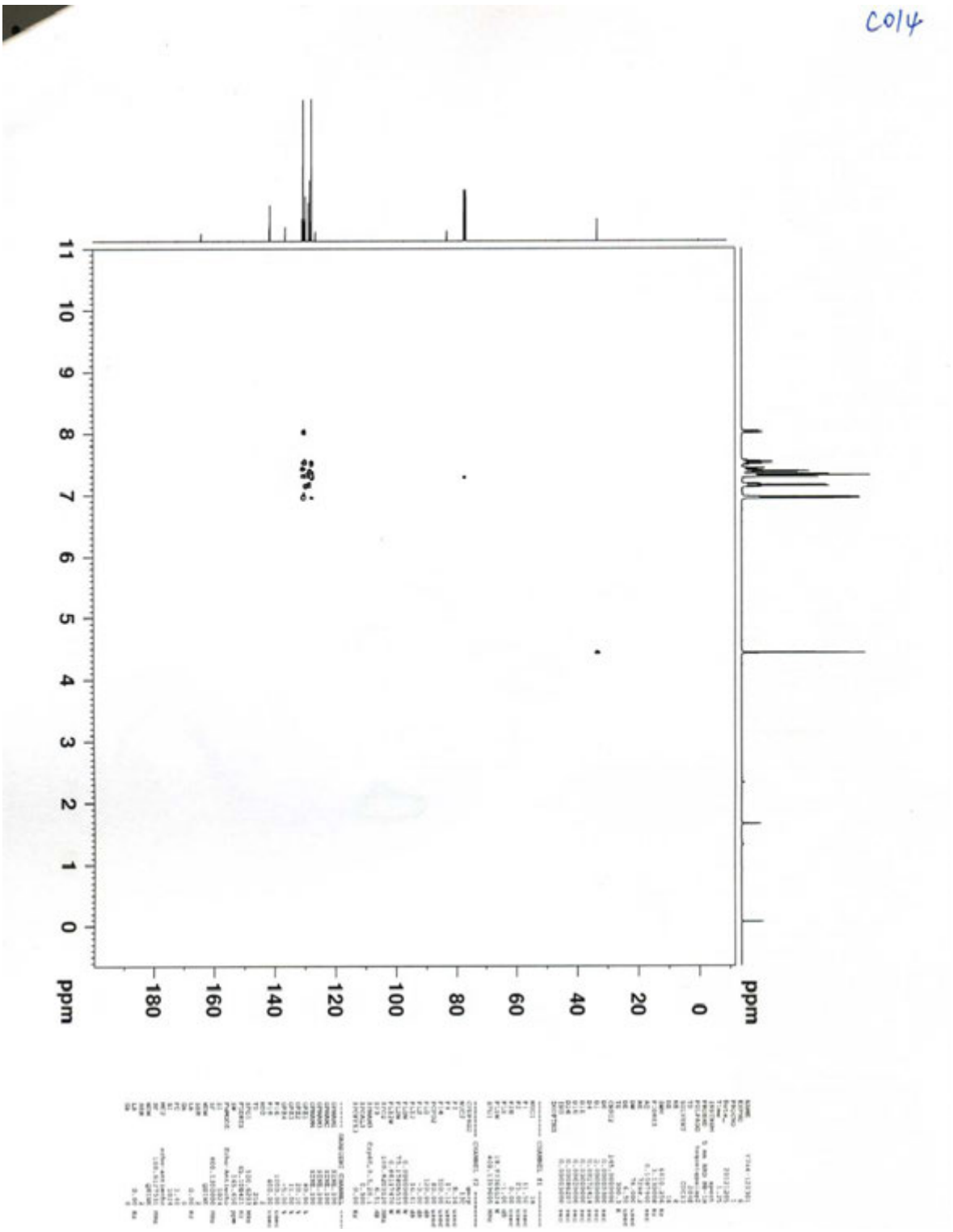

Fig 3.2.S.2.3-29 HSQC spectrum of BBTT

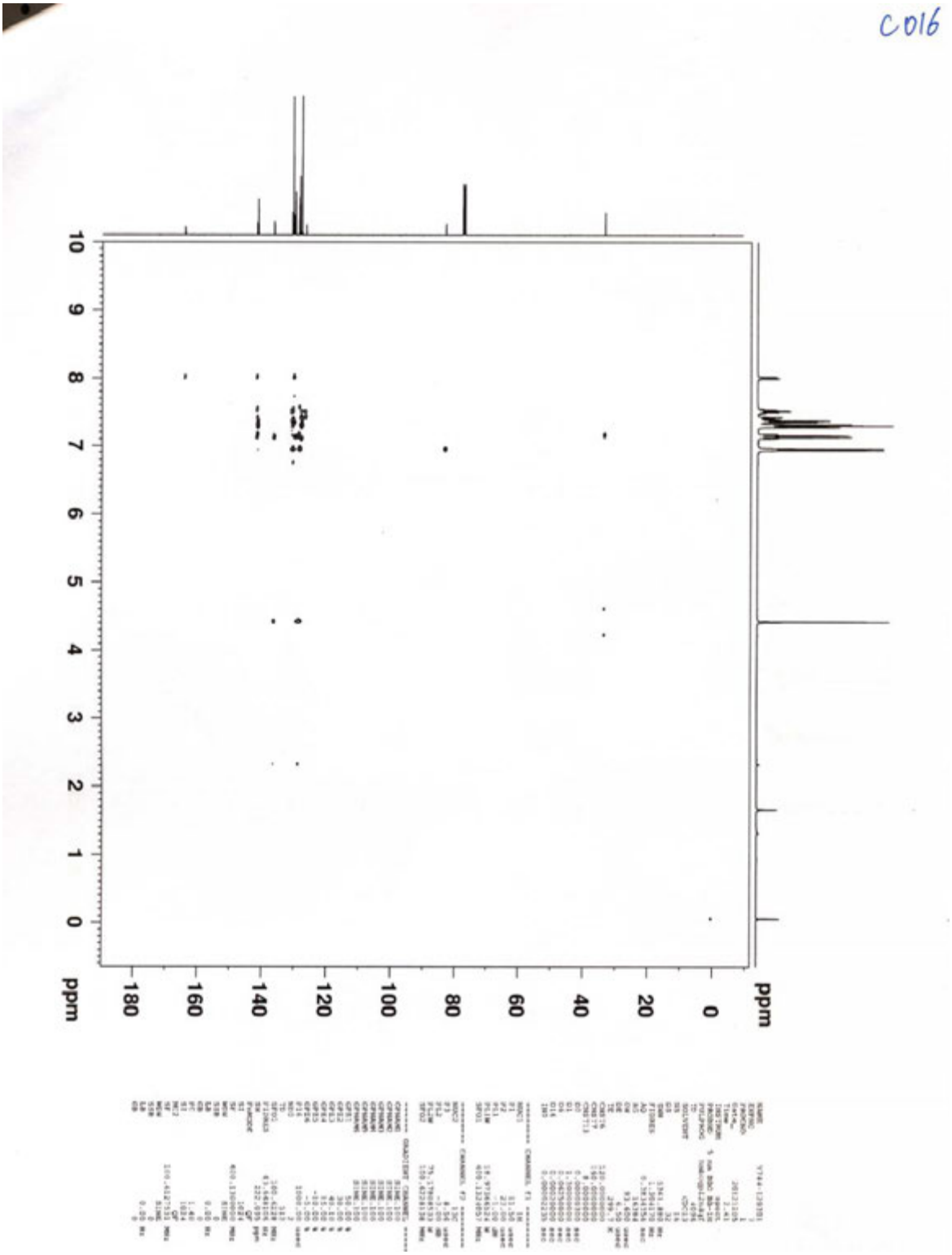

Fig 3.2.S.2.3-30 HMBC spectrum of BBTT

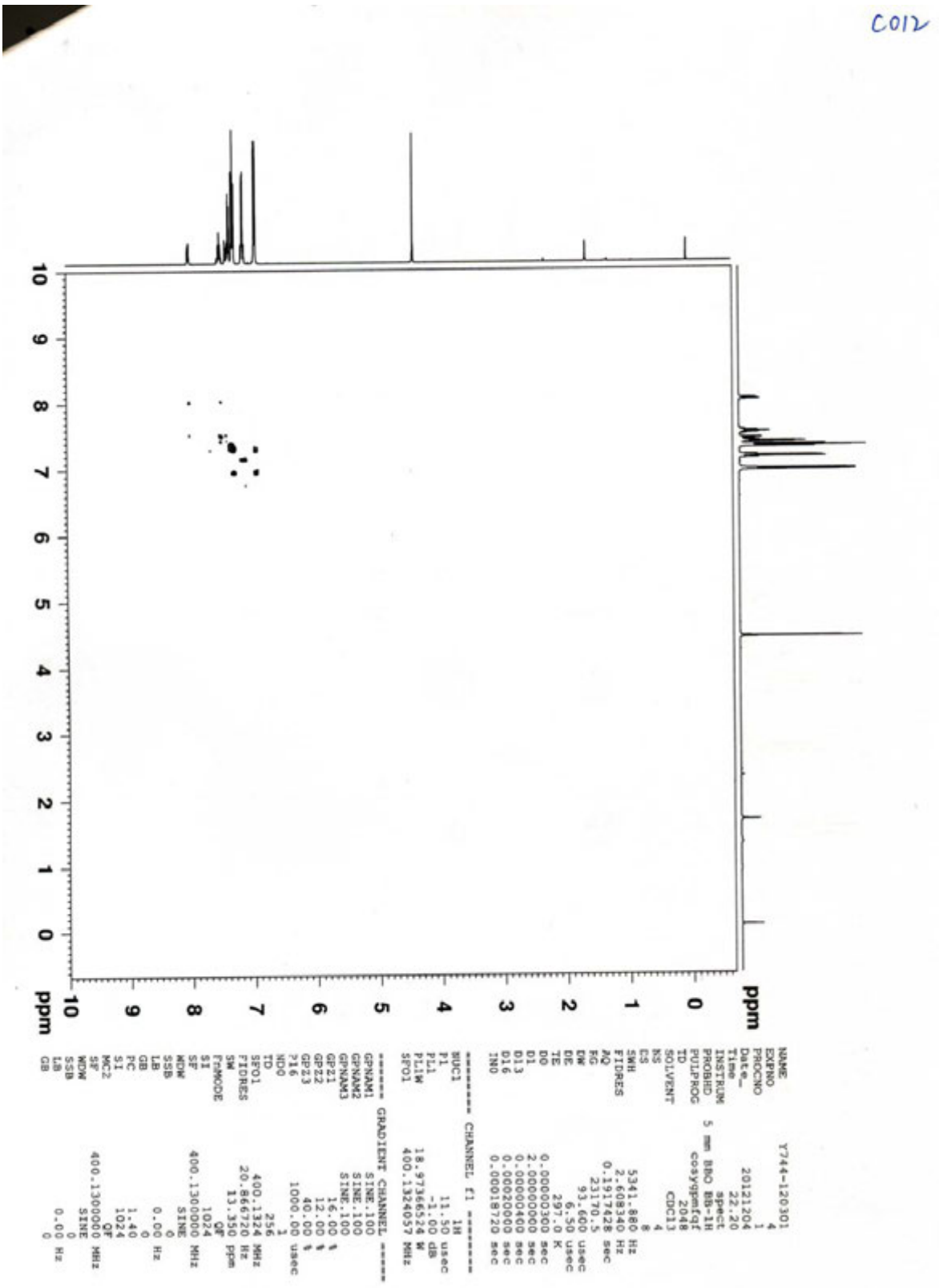

Fig 3.2.S.2.3-31 ^{1}H-^{1}H COSY spectrum of BBTT

3.2.S.2.3.3 Specification for the Reagents and Solvents

Every incoming batch of reagents and solvents used in manufacturing Irbesartan is accepted based on the in-house tests to ensure it meets the acceptance criteria according to the interal specification. The specification of the reagents and solvents are listed in the following tables.

Table 3.2.S.2.3-12 Specification for Tetrabutylammonium Hydrogen Sulfate (Code: Y742)

Molecular Formula: $C_{16}H_{36}N \cdot HSO_4$ **Molecular Weight:** 339.54			
Test		**Acceptance Criteria**	**Analytical Method**
Appearance		White crystal or crystalline powder	Visual examination
Identification	1	Should be a positive reaction	Chemical reaction
	2	Should be a positive reaction	
Assay*		Not less than 95.0%	Titration

*: assay is not a test item, it is a confirmation item according to the test result provided by supplier.

Table 3.2.S.2.3-13 Specification for Solid Sodium Hydroxide (Code: Y030)

Molecular Formula: NaOH **Molecular Weight:** 40.00		
Test	**Acceptance Criteria**	**Analytical Method**
Appearance	White or slightly colored solid with a lustrous surface	Visual examination
Identification	Should be a positive reaction	Chemical reaction
Assay (NaOH %)	Not less than 94.0.0%	Titration
Na_2CO_3	Not more than 1.7%	Titration

Table 3.2.S.2.3-14 Specification for Hydrochloric Acid (Code: Y025)

Molecular Formula: HCl **Molecular Weight:** 36.46			
Test		**Acceptance Criteria**	**Analytical Method**
Appearance		Transparent, colorless or light yellow liquid	Visual examination
Identification	1	Should be a positive reaction	Chemical reaction
	2	Should be a positive reaction	
Total acidity (Calculate as HCl)		Not less than 25.0%	Titration

Table 3.2.S.2.3-15 Specification for Anhydrous Ethanol (Code: Y050)

Molecular Formula: C_2H_6O **Molecular Weight:** 46.07		
Test	**Acceptance Criteria**	**Analytical Method**
Appearance	Colorless and transparent liquid	Visual examination
Identification	The retention time of the main peak obtained with the sample agrees with that of the reference.	GC
Water	Not more than 0.7%	Karl Fischer
Assay (EtOH %)	Not less than 99.0%	GC
Content of Methanol	Not more than 0.5%	
Content of *iso*-propanol	Not more than 0.1%	

Table 3.2.S.2.3-16 Specification for Toluene
(Code*: Fersh:Y049, Recovered: RY049)

Molecular Formula: C_7H_8 **Molecular Weight:** 92.14			
Test	**Acceptance Criteria**		**Analytical Method**
	Y049	**RY049**	
Appearance	Transparent liquid and no water-insoluble impurity	Transparent liquid	Visual examination
Identification	The retention time of the principal peak obtained with the sample should be consistent with that of the reference.		GC
Density (20°C)	860-875 kg/m^3	-	Hydrostatic method
Water	Not more than 1.0%	Not more than 3.0%	Karl Fisher
Benzene	Not more than 0.1%	-	GC
Any other impurities	Not more than 2.0 %	-	
Assay	Not less than 95.0%	Not less than 95.0%	GC

Table 3.2.S.2.3-17 Specification for Activated Carbon (Code: Y064)

Test	Acceptance Criteria	Analytical Method
Appearance	A black powder, odorless and tasteless; without grittiness.	Visual examination
Identification	Should be a positive reaction	Chemical reaction
pH Value	The filtrate should be clear and appear neutral when tested with litmus paper.	Chemical reaction
Chlorides	Not more than 0.1%	Colorimetry
Sulfates	Not more than 0.05%	Colorimetry
Non-carbonized Substance	The test solution should be not more intensely colored than the Standard Solution.	Colorimetry
Acid-soluble Substances	Not more than 10 mg	Weighing
Loss on Drying	Not more than 10.0%	Drying
Residue on Ignition	Not more than 3.0%	Weighing
Iron salt	Not more than 0.05%	Colorimetry
Zinc salt	Not more than 0.02%	Colorimetry
Heavy Metals	Not more than 30 ppm	Colorimetry
Adsorption Capacity	No turbidity	Chemical reaction
	The different volume of iodine VS consumed by sample and blank should be not less than 1.2 mL	Titration

The potable water used in the synthesis is tested following Chinese national standard GB5749-85 Specification and analytical procedure of potable water which is consistent with WHO guideline for Potable water quality.

3.2.S.2.3.4 Analytical Procedures for the Reagents and Solvents

1. Tetrabutylammonium Hydrogen Sulfate (Code: Y742)

Appearance

It is a white crystal or crystalline powder by visual examination.

Identification

1. Dissolve suitable amount of the sample with water. Add barium chloride and then precipitation can be observed. Add nitric acid and the precipitation does not dissolve.
2. Take suitable amount of the sample and add excessive amount of sodium hydroxide solution, heat and the gas generated is pungent which can turn wet red litmus paper to blue.

2. Solid Sodium Hydroxide (Code: Y005)

Appearance

It is a white or slightly colored solid with a lustrous surface by visual examination.

Identification

Transfer about 0.3 g of the sample to a 250mL conical flask. Add 150 mL of water, 10 mL of 10% barium chloride solution and then 2 - 3 drops of phenolphthalein indicator. The solution should be pink.

Assay (NaOH %)

Reagents

Hydrochloric acid VS (1.0 mol/L)
Phenophthalein indicator
10% barium chloride solution

Procedure

Rapidly weigh 36 ± 1 g (accurately to 0.01 g) of the sample, and place in a 1000-mL volumetric flask containing 300 mL of water. Dilute close to the volume and cool to room temperature. Dilute to volume and mix well to obtain the test solution.

Take 50 mL of the test solution and transfer to a 250-mL ground glass-stoppered conical flask. Add 10 mL of 10% barium chloride solution and 2 ~ 3 drops of phenolphthalein indicator. Titrate with hydrochloric acid VS (1.0 mol/L) until the solution becomes reddish. 1 mL of hydrochloric acid VS (1.0 mol/L) is equivalent to 40.00 mg of sodium hydroxide.

Calculation

$$X = \frac{C(V - V_0) \times 0.04000}{m \times 50 \div 1000} \times 100\%$$

In which
C - Actual concentration of hydrochloric acid VS, mol/L;
V - Volume of hydrochloric acid VS consumed by the sample, mL;
m - Weight of sample, g;
0.04000 - 1 mL of hydrochloric acid VS (1.0 mol/L) is equivalent to the mass (g) of sodium hydroxide, g/mol;

Test two samples in parallel and use the arithmetical mean of the two results as the final result. The absolute deviation between parallel tests should not exceed 0.2%.

Content of Sodium carbonate

Reagents

Hydrochloric acid VS (1.0 mol/L)
Bromocresol green-methyl red indicator

Procedure

Rapidly weigh 36 ± 1 g (accurately to 0.01 g) of the sample and place in a 1000-mL volumetric flask containing 300 mL of water. Dilute to close to the volume and cool to room temperature. Dilute to volume and mix well to obtain the test solution.

Take 50mL of the test solution and transfer to a 250-mL ground glass-stoppered conical flask. Add 10 mL of 10% bromocresol green-methyl red indicator and titrate with hydrochloric acid VS (1.0 mol/L) until the solution becomes wine red. The hydrochloric acid consumed here minus that consumed by the sodium hydroxide is that consumed by the sodium carbonate. 1 mL of hydrochloric acid VS (1.0 mol/L) is equivalent to 52.99 mg of sodium carbonate.

Calculation

$$X = \frac{C(V - V_0) \times 0.05299}{m \times 50 \div 1000} \times 100\%$$

In which
C - Actual concentration of hydrochloric acid VS, mol/L;
V - Volume of hydrochloric acid VS consumed by sodium hydroxide and sodium carbonate, mL;
V - Volume of hydrochloric acid VS consumed by sodium hydroxide, mL;
m - Weight of sample, g;
0.05299 - 1 mL of hydrochloric acid VS (1.0 mol/L) is equivalent to the mass (g) of sodium carbonate, g/mol;

Test two samples in parallel and use the arithmetical mean of the two results as the final result. The absolute deviation between parallel tests should not exceed 0.1%.

3. Hydrochloric Acid (Code: Y025)

Appearance

It is a transparent, colorless or light yellow liquid without visible impurity.

Identification

Procedure

(1) Transfer about 3 drops sample into 5ml water and then add silver nitrate TS, it should produce a white curdy precipitate that is insoluble in nitric acid but is soluble in a slight excess of ammonium hydroxide.
(2) Add 10 ml water into 1ml of sample, then add 3 drops methyl red indicator, the solution should be red.

Acidity (calculated as hydrochloric acid)

Reagent and Solution

Sodium hydroxide VS (1.0 mol/L)
Bromocresol green (1 g/L ethanol solution) indicator

Procedure

Add about 3 mL of the sample to an accurately weighed (accurate to 0.0002 g) 15mL conical flask, which contains 15 mL water. Mix and weigh (accurate to 0.0002 g). Add 2 ~ 3 drops of bromocresol green (1 g/L ethanol solution) indicator. Titrate the solution with 1.0 mol/L sodium hydroxide VS until the solution becomes blue, which indicates the end-point. 1.0 mL of sodium hydroxide VS is equivalent to 0.03646 g of hydrochloric acid.

Calculation

Calculate the acidity as hydrochloric acid (%)

$$X = \frac{C \times V \times 0.03646}{m} \times 100\%$$

In which,
V - Volume of Sodium hydroxide VS consumed to titrate the sample, mL;
C - Actual concentration of sodium hydroxide VS, mol/L;
m - Weight of the sample, g;

Test two samples in parallel and use the arithmetical mean of the two results as the final result. The relative deviation between parallel tests should not exceed 0.5%.

4. Anhydrous ethanol (Code: Y050)

Appearance

It is a colorless and transparent liquid by visual examination.

Identification

Examine the chromatogram obtained by GC. The retention time of the main peak should agree with that of the reference.

Water

Reagents

Anhydrous methanol
Karl Fischer reagent obtained commercially

Procedure

Take 5 mL of the sample and proceed as directed in the China Pharmacopoeia Appendix VIII M method I. The water content should be not more than 0.7%.

Test two samples in parallel. The absolute deviation of the two results should be not more than 0.05%. Calculate the arithmetical mean value of the two results as the test result.

Assay

Reagents
Nitrogen
Anhydrous ethanol

Chromatographic Conditions

Column	DB-624 (30 m×0.53 mm, 3.0 μm)
Carrier gas	Nitrogen
Pressure	0.4 MPa
Oven	Maintain the temperature at 50°C for 2 min. Raise it to 100°C a rate of 10 °C /min and hold at 100°C for 5 min.
Injection volume	0.2 μL
Detector	FID detector at 300°C
Injection port	250°C

Procedure

Inject 0.2 μL of the sample into the chromatograph and record the chromatogram. Calculate the assay by dividing the main peak area into the total peak area (excluding the water peak).

Repeat the injection and calculate the arithmetical mean value of the two results as the test result. The absolute deviation of the two results should be not more than 0.2%.

Content of Methanol

Proceed as directed in *Assay*. Inject methanol (AR) into the chromatograph and record the chromatogram as the reference, in which the retention time of methanol is 3.4 min. Identify the methanol peak in the chromatogram obtained using a sample and calculate the content of methanol by using the Area Normalization Method.

Content of *iso*-propanol

Proceed as directed in *Assay*. Inject *iso*-propanol (AR) into the chromatograph and record the chromatogram as the reference, in which the retention time of *iso*-propanol is 4.6 min. Identify the *iso*-propanol peak in the chromatogram obtained using a sample -nd calculate the content of *iso*-propanol by using the Area Normalization Method.

5. Toluene (Code: Y049&RY049)

Appearance

It is a transparent liquid without water-insoluble and mechanical impurities by visual examination.

Identification

Examine the chromatogram obtained in the *Assay*. The retention time of the principal peak in the sample GC chromatogram should be consistent with that of the reference (Toluene *AR*).

Density

Place the well mixed sample in a clean and dry measuring cylinder. Place a densitometer in it when the temperature of sample reaches 20±5°C. Record the temperature after the temperature stabilizes. Record the density (ρ_t) at the base of the meniscus. Avoid creating air bubbles in the sample and ensure that the end of the densiometer does not contact the wall of the measuring cylinder. Calculate the density at 20°C using the following formula:

$$\rho_{20} = \rho_t + r(t - 20)$$

In which,

ρ_{20} - Density of the sample at 20°C;

ρ_t - Visual density of the sample at the testing temperature;

t - Temperature during testing (°C);

r - 0.92 kg/m^3/°C.

Test two samples in parallel and use the arithmetical mean of the two results as the final result. The absolute difference between parallel tests should not exceed 2.0.

Water

Accurately weigh 0.5-1.0 g of the sample and titrate it with Karl Fischer reagent. Test two samples in parallel and use the arithmetical mean of the two results as the final result. The absolute deviation between parallel tests should not exceed 0.05%.

Assay

Chromatograph Conditions

Column:	DB-624, 30 m*0.53 mm*3 μm
Carrier gas:	Nitrogen
Flow:	3.0 mL/min (constant flow)
Oven:	Maintain at 40°C for 2 min. Raise temperature from 40°C to 120°C at a rate of 15°C /min, maintain at 120°C for 15 min.
Injection:	0.5 μL (inject directly)
Split ratio:	40:1
Detection:	FID at 250°C

Injection port: 200°C

Procedure

Inject the sample and toluene *AR* into the chromatograph and record the chromatograms. The content of toluene is obtained by subtracting the water content from the percentage of principal peak calculated using the area normalization method.

Benzene

Examine the chromatogram obtained in the *Assay*. Using the same test conditions, inject benzene *AR* and record the chromatogram. Identify the benzene peak in the sample GC chromatogram and calculate the percentage of benzene using the area normalization method.

Total Impurities (except for benzene)

Examine the chromatogram obtained in the *Assay*. Calculate the percentage of total impurities (except for benzene) using the area normalization method.

6. Activated Carbon (Code: Y064)

Appearance

It is a black, odorless and tasteless powder without grittiness by visual examination.

Identification

Procedure

Take 0.1 g of the sample and place in a heat-resisting glass tube. Slowly introduce compressed air into the tube and at the same time, heat the tube at the position of the sample using an alcohol burner, being careful to avoid ignition. The gas generated is introduced into a calcium hydroxide solution where it causes a white precipitate to appear.

pH value

Procedure

Take 2.5 g of the sample, add 50 mL of water and boil for 5 min. Cool, filter and wash

the residue with water. Combine the filtrate and the eluate to obtain 50 mL of liquid. The liquid should be clear and neutral when tested with litmus paper.

Chlorides

Procedure

Dilute 10 mL of the filtrate from the pH test with 200 mL of water and mix well. Take 20 mL , add 10 mL of diluted nitric acid and dilute with water to 40 mL. Mix well to obtain the test solution. Accurately measure 5.0 mL of standard sodium chloride solution (1 mL is equivalent to 10 μg of chlorine) and place in a 50-mL color-comparison tube. Add 10 mL of nitric acid and dilute with water to 40 mL. Mix well to obtain the reference solution. Add 1.0 mL of silver nitrate to both the test and reference solution. Dilute both solutions with water to 50 mL, mix well and allow to stand in a dark place for 5 min. Examine both solutions against a black background. The color of the test solution should not be deeper than that of the reference solution (0.1%).

Sulfate

Procedure

Take 20 mL of the filtrate from the test of pH value and dilute with water to 40 mL. Proceed as directed in China Pharmacopoeia Appendix VIII B. Place in a 50-mL Nessler tube, add 2 mL of diluted hydrochloric acid and mix well to obtain the test solution. Prepare the reference solution by transferring 5.0 mL of standard potassium sulfate solution (1 mL is equivalent to 100 μL of sulfate) to a 50-mL Nessler tube, adding water to about 40 mL and 2 mL of dilute hydrochloric acid and mixing well. Add 5 mL of 25% barium chloride solution to both the test solution and reference solution, dilute them with water to 50 mL, mix well and allow to stand for 10 min. Observe both solutions against a dark background. The colour of the test solution should not be more intense than that of the reference solution (0.05%).

Non-carbonized Substance

Procedure

Take 0.25 g of the sample, add 10 mL of sodium hydroxide, boil and filter to obtain the test solution. If the filtrate is coloured, the colour should not be more intense than that of the reference solution which consists of 0.3 mL of cobalt chloride CS, 0.2 mL of potassium dichromate CS and 9.5 mL of water.

Acid-soluble Substances

Procedure

Take 1.0 g of the sample, add 20 mL of water and 5 mL of hydrochloric acid. Boil for 5 min, and filter. Wash the residue with 10 mL of hot water and combine the filtrate and eluate. Add 1 mL of sulfuric acid and evaporate to dryness. Ignite to constant weight. The residue should not weigh more than 10 mg.

Calculation

$$X = \frac{m_1 - m_0}{m} \times 1000$$

In which
m_1 - Total weight of crucible and residue after ignition at 700 ~ 800°C, g;
m_0 - Weight of empty crucible after ignition at 700 ~ 800°C, g;
m - Weight of the sample, g.

Test two samples and use the arithmetical mean of the two results as the final result. The absolute deviation between parallel tests should not exceed 0.05%.

Loss on Drying

Procedure

Take the sample and dry at 120°C to constant weight. The weight loss should not be more than 10.0% (China Pharmacopoeia Appendix VIII L).

Calculation

$$X = \frac{M_0 + m - M}{m} \times 100\%$$

In which
M_0 - Weight of empty weighing bottle after drying at 120°C, g;
M - Total weight of weighing bottle and sample after drying at 120°C, g;
m - Weight of the sample, g.

Test two samples and use the arithmetical mean of the two results as the final result. The absolute deviation between parallel tests should not exceed 0.2%.

Residue on Ignition

Procedure

Weigh about 0.5 g of the sample and add 2 ~ 3 drops of ethanol to wet it. Proceed as directed in China Pharmacopoeia Appendix VIII N. The residue on ignition should not be more than 3.0%.

Calculation

$$X = \frac{M - M_0}{m} \times 100\%$$

In which
M_0 - Weight of empty crucible after ignition at 700 ~ 800°C, g;
M - Total weight of crucible and sample after ignition at 120°C, g;
m - Weight of the sample, g.

Test two samples and use the arithmetical mean of the two results as the final result. The absolute deviation between parallel tests should not exceed 0.1%.

Iron Salt

Procedure

Take 1.0 g of the sample, add 25 mL of 1 mol/L hydrochloric acid, boil for 5 min and allow to cool. Filter and wash the residue repeatedly with aliquots of hot water which together do not exceed a total of 30 mL. Combine the filtrate and the eluate, add water to 100 mL and mix well. Pipette 5 mL of the solution into a 50-mL color-comparison tube, add 4 mL of dilute hydrochloric acid and 50 mg of ammonium persulfate. Dilute with water to 35 mL and proceed as directed in China Pharmacopoeia Appendix VIII G. Add 3 mL of 30% ammonium thiocyanate solution, followed by water to 50 mL. Mix well to obtain the test solution. Prepare a reference solution by accurately pipetting 2.5 mL of standard iron solution (1 mL is equivalent to 10 μg of iron) into a 50 mL color-comparison tube, adding water to 25 mL, followed by 4 mL of dilute hydrochloric acid and ammonium persulfate. Dilute with water to 35 mL, add 3 mL of 30% ammonium thiocyanate solution and water to 50 mL before mixing well. Compare the solutions. The colour of the test solution should not be more intense than that of the reference solution (0.05%).

Zinc Salt

Procedure

Take 1.0 g of the sample and add 25 mL of water. Boil for 5 min, cool and filter. Filter and wash the residue repeatedly with aliquots of water which together do not exceed a total of 30 mL. Combine the filtrate and eluate, add water to 100 mL and mix well. Accurately pipette 10 mL into a 50 mL Nessler tube, add 0.5 g of ascorbic acid, 4 mL of hydrochloric acid (1→2) and 3 mL of potassium ferrocyanide. Dilute with water to volume and mix well to obtain the test solution. If the solution is turbid, prepare the standard zinc solution (preparation: accurately weigh 44 mg of zinc sulfate, place in a 100-mL volumetric flask, add water to dissolve and dilute to volume before mixing well. Accurately pipette 10 mL into a 100-mL volumetric flask, dilute with water to volume and mix well to obtain the standard zinc solution. 1 mL of standard zinc solution is equivalent to 10 μg of zinc.). Transfer 2 mL of standard zinc solution to a 50-mL Nessler tube. Add 0.5 g of ascorbic acid, 4 mL of hydrochloric acid (1→2) and 3 mL of potassium ferrocyanide. Dilute with water to volume and mix well to obtain the reference solution. Compare the solutions. The color of the test solution should not be more intense than that of the reference solution (0.02%).

Heavy Metals

Procedure

Take 1.0 g of the sample, add 10 mL of hydrochloric acid and 5 mL of bromine solution. Boil for 5 min and filter. Wash the residue with 35 mL boiling water. Combine the filtrate and eluate, add water to 50 mL and mix well. Take 20 mL, add 1 drop of phenolphthalein indicator, and add ammonia solution dropwise until the color of solution changes to light red. Add 2 mL of acetate buffer (pH 3.5) and sufficient water to 25 mL. Add 0.5 g of ascorbic acid and allow to dissolve to obtain the test solution. Prepare a reference solution by pipetting 1.2 mL of standard lead solution (1 mL is equivalent to 10 μg of lead), adding 2 mL of acetate buffer (pH 3.5) and sufficient water to 25 mL. Add ascorbic acid and allow to dissolve to provide the reference solution. Add 2 mL of thioacetamide solution to both the test solution and reference solution. Mix well. After 5 min, observe the two solutions against the same white paper. The color of the test solution should not be more intense than that of the reference solution. The content of heavy metal should not be more than thirty millionths.

Adsorption Capacity

Procedure

1) Take 1.0 g of the sample which has been previously dried to constant weight and add 100 mL of 0.12% quinine sulfate solution. Shake for 5 min at room temperature (not lower than 20°C) and filter immediately through a mid-speed filter paper. Take 10 mL of the subsequent filtrate, add 1 drop of hydrochloric acid and 5 drops of potassium mercuric iodide solution. The solution should not become turbid.
2) Accurately pipette 50 mL of 0.1% methylene blue solution into each of two 100-mL measuring cylinders with stoppers. To one of the cylinders, add 0.25 g of the sample, which has been dried to constant weight, close tightly and shake vigorously at room temperature (not lower than 20°C) for 5min. Filter the solutions in the two cylinders with mid-speed filter papers. Transfer 25 mL of each filtrate to separate 250-mL measuring flasks and add 50 mL of 10% sodium acetate to each one. Mix well. Accurately add 35 mL of iodine VS (0.05 mol/L) to each flasks, close tightly and mix well. Shake vigorously every 10 min. After 50 min, dilute with water to volume, and allow to stand for 10 min. Filter both solutions through dry filter paper. Accurately titrate 100 mL of each filtrate with sodium thiosulfate VS (0.1 mol/L). The difference in the volumes of iodine VS consumed by the two solutions should be not less than 1.2 mL.

Calculation

$$X = \frac{C(V - V_0)}{0.05 \times 2 \times m} \times 0.25$$

In which
C - Actual concentration of sodium thiosulfate VS, mol/L;
V_0 - Volume of sodium thiosulfate VS consumed in blank determination, mL;
V - Volume of sodium thiosulfate VS consumed by sample, mL;
m - Weight of sample, g.

Test two samples and use the arithmetical mean of the two results as the final result.