SN

中华人民共和国出入境检验检疫行业标准

SN/T 1605—2005

进出口植物性产品中氰草津、氟草隆、莠去津、敌稗、利谷隆残留量检验方法 高效液相色谱法

Inspection of cyanazin, fluometuron, atrazine, propanil and linuron residues in products of plant origin for import and export—HPLC

2005-08-18 发布　　2006-02-01 实施

中华人民共和国
国家质量监督检验检疫总局　发布

前　言

本标准附录A和附录B为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准由中华人民共和国上海出入境检验检疫局负责起草。

本标准主要起草人：郭德华、李波、俞秋蓉、韩丽、王敏、王传现、王东辉、魏玉璞。

本标准系首次发布的出入境检验检疫行业标准。

进出口植物性产品中氰草津、氟草隆、莠去津、敌稗、利谷隆残留量检验方法 高效液相色谱法

1 范围

本标准规定了进出口粮谷中氰草津、氟草隆、莠去津、敌稗、利谷隆残留量的抽样、制样和测定方法。

本标准适用于进出口小麦、大麦、大豆、油菜籽和大米中氰草津、氟草隆、莠去津、敌稗、利谷隆残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 4 000 袋为一检验批。

同一检验批的商品应具有相同的特征，如包装、标记、产地、规格和等级等。

2.2 抽样数量

按式(1)确定抽样数量。

$$a = \sqrt{N} \qquad \cdots\cdots(1)$$

式中：

N——全批袋数；

a——抽样袋数。

注：a 值取整数，小数部分向前进位为整数。

2.3 抽样工具

2.3.1 金属单管取样器：不锈钢管，全长 55 cm(包括手柄)，直径 1.5 cm，沟槽长度应超过袋对角线长度的一半。

2.3.2 取样铲。

2.3.3 分样板。

2.3.4 样品筒(袋)：可密封。

2.3.5 分样布或适用铺垫物。

2.4 抽样方法

2.4.1 倒包抽样

从堆垛的各部位随机抽取 2.2 规定的应抽样件数的 10%(每批一般不少于 3 袋)，将袋口缝线全部拆开，平置于分样布或其他洁净的铺垫物上，双手紧握袋底两角，提起约成 45°倾角，倒拖约 1 m，使袋内货物全部倒出。查看袋内和袋间品质是否均匀。确认情况正常后，用取样铲随机在各部位抽取样品，立即将样品倒入盛样器内。每袋抽取样品数量应基本一致。

2.4.2 袋内抽样

按 2.2 规定的应抽样袋数的 90%，在堆垛四周上、中、下各层以曲线形走向随机抽取。将取样器(2.3.1)管槽朝下，从每袋一角依斜对角方向插入袋内，然后将管槽旋转朝上，抽出取样器，立即将样品倒入盛样器内。每袋抽取样品数量应与 2.4.1 基本一致。

2.4.3 大样缩分

集中倒包抽样和袋内抽样所取全部样品，倒于分样布上，用分样板按四分法缩分出样品不少于

2 kg，盛于样品筒内，加封后，标明标记并及时送交实验室。

2.5 试样制备

将样品按四分法缩分出约 1 kg，全部磨碎并通过 20 目筛，混匀，均分成两份试样，装入洁净的容器内，密封，标明标记。

2.6 试样保存

将试样于－5℃以下避光保存。

3 测定方法

3.1 方法提要

试样中的残留物用乙腈提取后过中性氧化铝小柱，浓缩后经固相萃取小柱净化，乙腈-水为流动相，高效液相色谱-质谱/质谱仪测定，用多反应监测(MRM)模式检测，外标法定量。

3.2 试剂和材料

除另有规定外，试剂均为液相色谱纯，水为重蒸馏水。

3.2.1 乙腈。

3.2.2 甲醇。

3.2.3 中性氧化铝：100 目～200 目，层析级。

3.2.4 氯草津标准品：纯度＞98%。

3.2.5 氟草隆标准品：纯度＞98%。

3.2.6 莠去津标准品：纯度＞98%。

3.2.7 敌稗标准品：纯度＞98%。

3.2.8 利谷隆标准品：纯度＞98%。

3.2.9 氯草津、氟草隆、莠去津、敌稗、利谷隆标准溶液：分别准确称取适量的氯草津、氟草隆、莠去津、敌稗、利谷隆标准品，用乙腈配成浓度为 1 mg/mL 的标准储备液。

3.2.10 氯草津、氟草隆、莠去津、敌稗、利谷隆标准混合溶液：准确移取适量上述标准储备液，用乙腈配成浓度为 10 μg/mL 的标准混合液。再以乙腈稀释成适用浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 高效液相色谱仪，配有四极杆串联质谱仪。

3.3.2 离心机：5 000 r/min。

3.3.3 高速均质器。

3.3.4 固相萃取装置。

3.3.5 筒型漏斗。

3.3.6 C_{18} 固相萃取小柱：6 mL、200 mg。

3.3.7 氮吹仪。

3.3.8 旋转蒸发仪。

3.4 测定步骤

3.4.1 提取

准确称取已研细样品 4.0 g 于 50 mL 离心管中，加 25 mL 乙腈后，用均质机以 10 000 r/min 的速度均质 5 min。以 2 000 r/min 的速度离心 15 min。上清液通过装有 5 g 中性氧化铝的筒型漏斗过滤至 100 mL 蒸发瓶中，滤渣再用 20 mL 乙腈振荡提取 5 min，重复上述操作，合并上清液于同一蒸发瓶中，于 50℃水浴减压浓缩至约 2.5 mL，加入 30 mL 水，混匀。

3.4.2 净化

在真空固相萃取装置上连接 C_{18} 固相萃取小柱，依次用 6 mL 甲醇、10 mL 水活化，将上述样液转移至 C_{18} 固相萃取小柱上，用 10 mL 10%的甲醇水溶液淋洗，弃去流出液。分析物用 5.0 mL 甲醇洗脱并

收集，洗脱液 40℃氮气流吹至近干。残留物用 1.0 mL 乙腈溶解，过 0.45 μm 微孔滤膜，滤液供液相色谱-质谱/质谱测定。

3.4.3 测定

3.4.3.1 液相色谱-质谱条件

a) 色谱柱：ODS-C_{18}（5 μm），250 mm×2.1 mm（内径）或相当者；
b) 流动相：乙腈-水（60+40）；
c) 流速：0.25 mL/min；
d) 柱温：室温；
e) 进样量：20 μL；
f) 电离方式：ESI+；
g) 毛细管电压：3.53 kV；
h) 锥体电压：31 V；
i) 离子源温度：100℃；
j) 干燥温度：420℃；
k) 雾化气：氮气，纯度＞99.9%，流量：67 L/h；
l) 干燥气：氮气，纯度＞99.9%，流量：376 L/h；
m) 碰撞能量：20 eV；
n) 测定方式：MRM；
o) 定性离子对和定量离子对见表 1。

表 1 定性离子对和定量离子对

除草剂名称	定性离子对(m/z)	定量离子对(m/z)
利谷隆	249/160 249/182	249/182
氟草隆	233/72 233/188	233/188
莠去津	216/174 216/96	216/96
敌稗	218/162 218/127	218/162
氰草津	241/214 241/132	241/132

3.4.3.2 液相色谱-质谱/质谱测定

根据样液中被测氰草津、氟草隆、莠去津、敌稗、利谷隆含量情况，选定峰高相近的标准工作溶液。标准工作溶液和样液中氰草津、氟草隆、莠去津、敌稗、利谷隆响应值均应在仪器检测线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述色谱条件下，氰草津、氟草隆、莠去津、敌稗和利谷隆的保留时间分别约为 4.03 min、4.80 min、5.57 min、7.34 min 和 8.38 min，标准品质谱图参见附录 A 中图 A.1 和附录 B 中图 B.1。

定性测定，样液如果检出的色谱峰的保留时间与标准溶液中某种除草剂相一致，并且所选择的子离子均出现，而且之间的丰度比也相一致，则可判定试样中含有该种除草剂。

3.4.4 空白试验

除不加试样外，均按上述测定步骤进行。

3.4.5 结果计算和表述

用色谱数据处理机或按式(2)计算样品中利谷隆、氟草隆、莠去津、敌稗、氯草津残留含量，计算结果应将空白值扣除。

$$X = \frac{A \times c \times V}{A_s \times m} \quad \cdots\cdots(2)$$

式中：

X——样品中氯草津、氟草隆、莠去津、敌稗、利谷隆的含量，单位为毫克每千克(mg/kg)；

c——氯草津、氟草隆、莠去津、敌稗、利谷隆标准工作液的浓度，单位为微克每毫升(μg/mL)；

A——样品中氯草津、氟草隆、莠去津、敌稗、利谷隆的峰面积；

A_s——氯草津、氟草隆、莠去津、敌稗、利谷隆标准工作液的峰面积；

V——样液最终定容体积，单位为毫升(mL)；

m——最终样液所代表的试样量，单位为克(g)。

4 测定低限、回收率

4.1 测定低限

本方法中氯草津、氟草隆、莠去津、敌稗、利谷隆的测定低限均为 0.01 mg/kg。

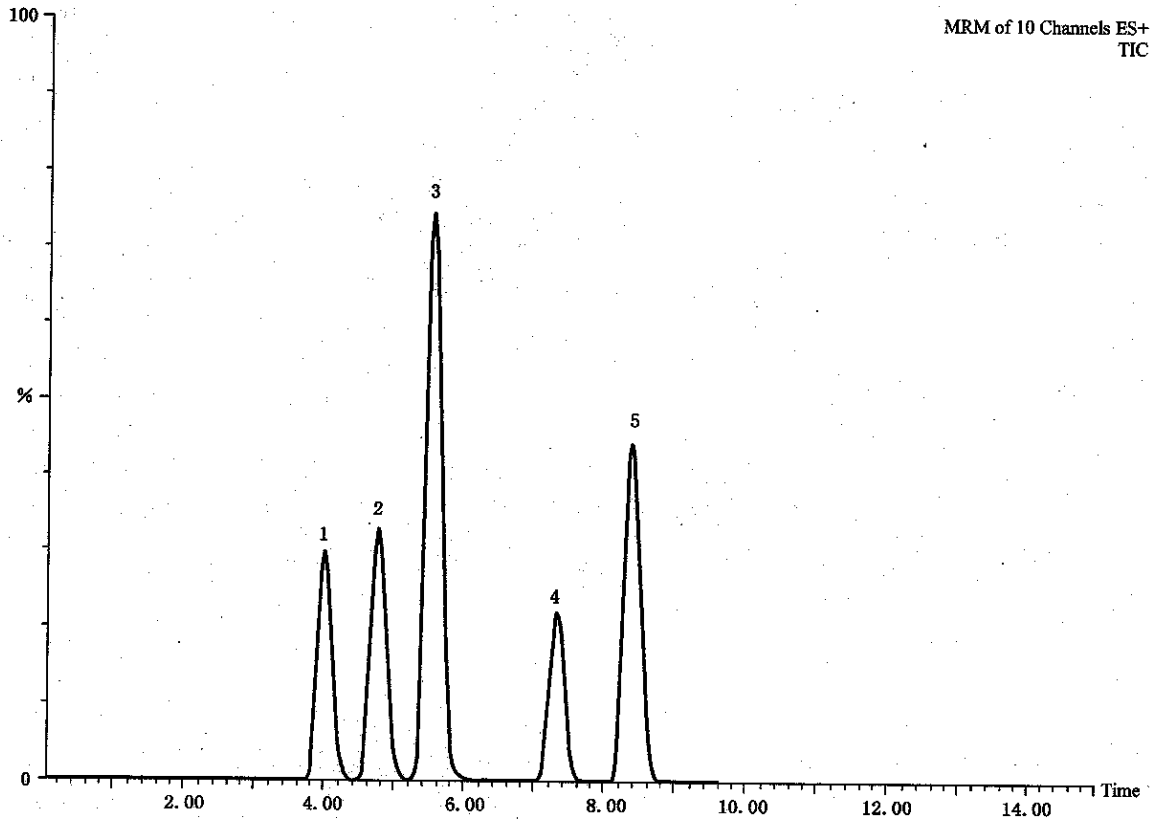
4.2 回收率

在小麦、大麦、大豆、油菜籽、大米中氯草津、氟草隆、莠去津、敌稗、利谷隆的添加浓度及其回收率实验数据见表 2。

表 2 实验数据表

除草剂名称	添加浓度/(mg/kg)	回收率范围
利谷隆	0.01 0.10 1.00	77.3%～88.5% 83.6%～98.6% 88.4%～102.5%
氟草隆	0.01 0.10 1.00	79.8%～89.5% 75.3%～92.2% 86.2%～98.7%
莠去津	0.01 0.10 1.00	79.5%～86.8% 77.8%～90.7% 80.5%～87.4%
敌稗	0.01 0.10 1.00	79.5%～86.9% 76.8%～90.6% 80.5%～95.4%
氯草津	0.01 0.10 1.00	80.6%～89.7% 83.9%～95.7% 80.5%～95.0%

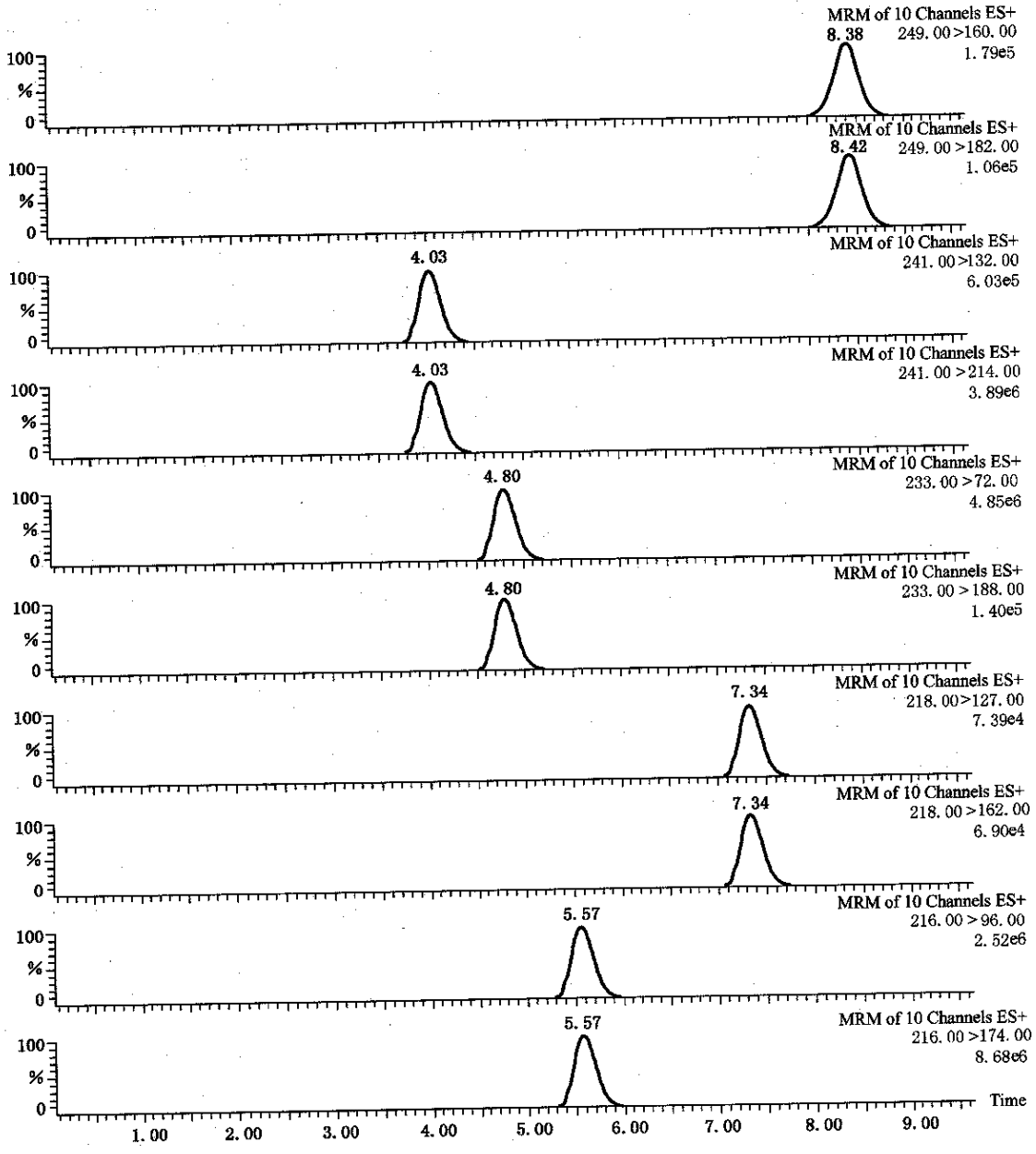
附 录 A
（资料性附录）
标准品 LC-MS/MS 总离子流图



注：色谱峰号 1,2,3,4,5 分别为氰草津、氟草隆、莠去津、敌稗和利谷隆。

图 A.1 氰草津、氟草隆、莠去津、敌稗、利谷隆的 TIC 图

附 录 B
（资料性附录）
标准品色谱图



注：氰草津、氟草隆、莠去津、敌稗和利谷隆的保留时间分别约为 4.03 min、4.80 min、5.57 min、7.34 min 和 8.38 min。

图 B.1 氰草津、氟草隆、莠去津、敌稗、利谷隆标准品的 MRM 图

Foreword

Annex A and annex B of this standard is an informative annex.

This standard was proposed by and is under the charge of the Certification and Accreditation Administration of the People's Republic of China.

This standard was drafted by Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Guo Dehua, Li Bo, Yu Qiurong, Han Li, Wang Min, Wang Chuanxian, Wang Donghui and Wei Yupu.

This standard is a professional standard of entry-exit inspection and quarantine promulgated for the first time.

Note: this English version, a translation from the Chinese text, is solely for guidance.

Inspection of cyanazin, fluometuron, atrazine, propanil and linuron residues in products of plant origin for import and export —HPLC

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of cyanazin, fluometuron, atrazine, propanil, linuron residues in plant origin products.

This standard is applicable to the determination of cyanazin, fluometuron, atrazine, propanil, linuron residues in wheat, barley, soybean, coleseed and rice for import and export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not exceed 4 000 packages.

The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification and grade, should be the same.

2.2 Quantity of sample taken

Sampling taken is according to the formula (1).

$$a = \sqrt{N} \qquad \cdots\cdots(1)$$

formula:

N——total number of bags in an inspection lot;

a——number of bags to be taken.

Note: If value a is with decimal, round off the decimal part, which is added as unity to the integral part of a.

2.3 Sampling tools

2.3.1 Metallic sampler: stainless steel; length (including handle): 55 cm; diameter: 1.5 cm; groove length: longer than half the diagonal length of the bag.

2.3.2 Sampling shovel

2.3.3 Plate for quartering

2.3.4 Sample container: Can or bag, which can be sealed.

2.3.5 Cloth (or other suitable material) sheet: For sample dividing (quartering)

2.4 Sampling procedure

2.4.1 Sampling by empting out

Draw 10 percent of the number of bags specified in 2.2 (not less than three bags) at any part of the pile at random. Unseal and open the bag, and place it on the sampling cloth sheet (or other clean sheet), Grasp tight two corners of the bag's bottom and raise up to an angle of 45 degree, tug backward for ca 1 m until all contents of the bag is emptied out. Check whether the quality of goods is uniform within and between the bags. After confirming the goods are in normal condition, scoop up the sample from different parts of the out-poured content at random, and promptly place in a clean sample container. The quantity of the sample drawn from each bag should be basically the same.

2.4.2 Sampling from inside the bags

Draw the samples from 90 percent of the number of bags specified in 2.2 (by deducting the numbet of bags drawn in 2.4.1.1). Along the sine wave of the pile, draw the samples from the bags of the upper, middle and lower parts around the pile at random. Insert the sampler, with its groove facing downward, diagonally into each bag, then turn the sampler by 180 degree, draw out the sampler, and promptly pour the sample into a container. The quantity of the sample drawn from each bag should be basically the same as in 2.4.1.

2.4.3 Reduction of gross sample

Pour all of samples on a clean sheet, reduce to not less than 2 kg with a plate by quartering. Place in a sample container, seal, label and sent to the laboratory in time.

2.5 Preparation of test sample

Reduce the sample to ca 1kg by quartering, grind thoroughly and let pass through a 20 mesh sieve, mix thoroughly and divide into 2 equal portions. Each portion is placed in a clean container as the test sample, seal and label.

2.6 Storage of test sample

The test samples should be stored below −5℃ and kept away from light.

3 Method of determination

3.1 Principle

Residues in sample were extracted with acetonitrile, and pass through a neutral aluminum oxide column. After concentrated, the solution is cleaned up by solid-phase extraction column. Determination is made by HPLC-MS/MS with the mobile phase of acetonitrile-water, more reaction monitor (MRM) model, external standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents used should be analytically pure. "Water" is redistilled water.

3.2.1 Acetonitrile: HPLC grade.

3.2.2 Methanol: HPLC grade.

3.2.3 Neutral aluminum oxide column: 100 mesh~200 mesh, for chromatography.

3.2.4 Cyanazin standard: Purity>98%.

3.2.5 Fluometuron standard: Purity>98%.

3.2.6 Atrazine standard: Purity>98%.

3.2.7 Propanil standard: Purity>98%.

3.2.8 Linuron standard: Purity>98%.

3.2.9 Cyanazin, fluometuron, atrazine, propanil, linuron standard solution: Accurately weigh an adequate amount of standard separately, dissolve in acetonitrile and prepare a solution of 1 mg/mL in concentration as the standard stock solution.

3.2.10 Cyanazin, fluometuron, atrazine, propanil, linuron standard mixture solution: Prepare a standard mixture solution of 10 μg/mL in concentration by diluting the above stock solutions with acetonitrile, then dilute to suitable concentration with acetonitrile as standard working solution.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph, equipped with quadrupole MS/MS tandem spectrometer.

3.3.2 Centrifuge: 5 000 r/min.

3.3.3 High-speed homogenizer.

3.3.4 Solid-phase extraction apparatus.

3.3.5 Cylinder filler.

3.3.6 Solid-phase extraction column: C_{18}, 6cc, 200 mg, or equivalent.

3.3.7 N_2 evaporator

3.3.8 Rotary evaporator

3.4 Procedure

3.4.1 Extraction

Weigh 4.0 g of the test sample grinded thoroughly into a 50 mL centrifuge tube. Add 25 mL acetonitrile and homogenize for 5 min at 10 000 r/min. Then centrifuge mixture at 2 000 r/min for 15 min. The supernatant is filtrated into a 100 mL evaporator by passing through a cylinder filler containing 5 g of neutral aluminum oxide. Add 20 mL acetonitrile to remanet in 50 mL centrifuge tube and repeat above procedure. Combine the supernatant to evaporator and concentrate it to the volume of 2.5 mL on a rotary evaporator at 50℃, adding 30 mL water and mixed solution.

3.4.2 Cleanup

Place solid-phase extraction column C_{18} onto solid-phase extraction apparatus and is conditioned with 6 mL methanol and 10 mL water. Pass the above solution through C_{18} column, wash with 10 mL 10% methanol-water and discard the effluents. Wash and collect the effluents with 5.0 mL methanol, and evaporate the effluents to dryness under a gentle stream of nitrogen at 40℃. The residue is resolved with 1.0 mL acetonitrile, filtered with 0.45 μm membrane and ready for HPLC-MS/MS determination.

3.4.3 Determination

3.4.3.1 HPLC-MS/MS operating conditions

a) Column: ODS-C_{18} (5 μm), 250 mm × 2.1 mm (i.d.) or equivalent;
b) Mobile phase: Acetonitrile-water (60 + 40);
c) Flow rate: 0.25 mL/min;
d) Column temperature: at room temperature;
e) Injection volume: 20 μL;
f) Electron ionization: ESI+;
g) Capillary voltage: 3.53 kV;
h) Cone voltage: 31 V;
i) Source temperature: 100℃;
j) Desolvation temperature: 420℃;
k) Cone gas: N_2, Purity>99.9%, 67 L/h;
l) Desolvation gas: N_2, Purity>99.9%, 376 L/h;
m) Collision energy: 20 eV;
n) Detection mode: MRM;
o) Qualitative ions and quantitative ions see table 1.

Table 1 Qualitative ions and quantitative ions

Herbicides	Qualitative ions (m/z)	Quantitative ions (m/z)
Cyanazin	249/160 249/182	249/182
Fluometuron	233/72 233/188	233/188
Atrazine	216/174 216/96	216/96
propanil	218/162 218/127	218/162
linuron	241/214 241/132	241/132

3.4.3.2 HPLC-MS/MS determination

According to the estimated approximate concentration of herbicides in the sample solution, select standard working solution of similar peak area to that of sample solution. The responses of herbicides in the standard working solution and the sample solution should be in the linear range of the instrumental detection. The standard working solution should be injected randomly in-between the injections of the sample solution of equal volume. Under the above chromatographic condition, the retention time of cyanazin, fluometuron, atrazine, propanil, linuron is 4.03 min, 4.80 min, 5.57 min, 7.34 min and 8.38 min respectively. For the chromatogram of standard, see fig. A.1 in annex A and fig. B.1 in annex B.

Confirmation: If there is a peak appeared at the same retention time for both of the sample solution and standard working solution, and all selected ions appeared and the ratio of abundance is according, confirm the herbicide being in the test sample.

3.4.4 Blank test

The operation of the blank test is the same as that described in the method of determination but with the omission of sample addition.

3.4.5 Calculation and expression of the result

The calculation of result is carried out by HPLC data processor or according to the formula (2), The blank value should be subtracted from the above result of calculation.

$$X = \frac{A \times c \times V}{A_s \times m} \quad \cdots\cdots (2)$$

where:

X——the residue content of cyanazin, fluometuron, atrazine, propanil, linuron in the test sample, mg/kg;

c——the concentratioin of cyanazin, fluometuron, atrazine, propanil, linuron in the standard working solution, μg/mL;

A——the peak area of cyanazin, fluometuron, atrazine, propanil, linuron in the sample solu-

tion;

A_s——the peak area of cyanazin, fluometuron, atrazine, propanil, linuron in the standard working solution;

V——the final volume of sample solution, mL;

m——the corresponding mass of the test sample in the final sample solution, g.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of cyanazin, fluometuron, atrazine, propanil, linuron is 10 μg/kg.

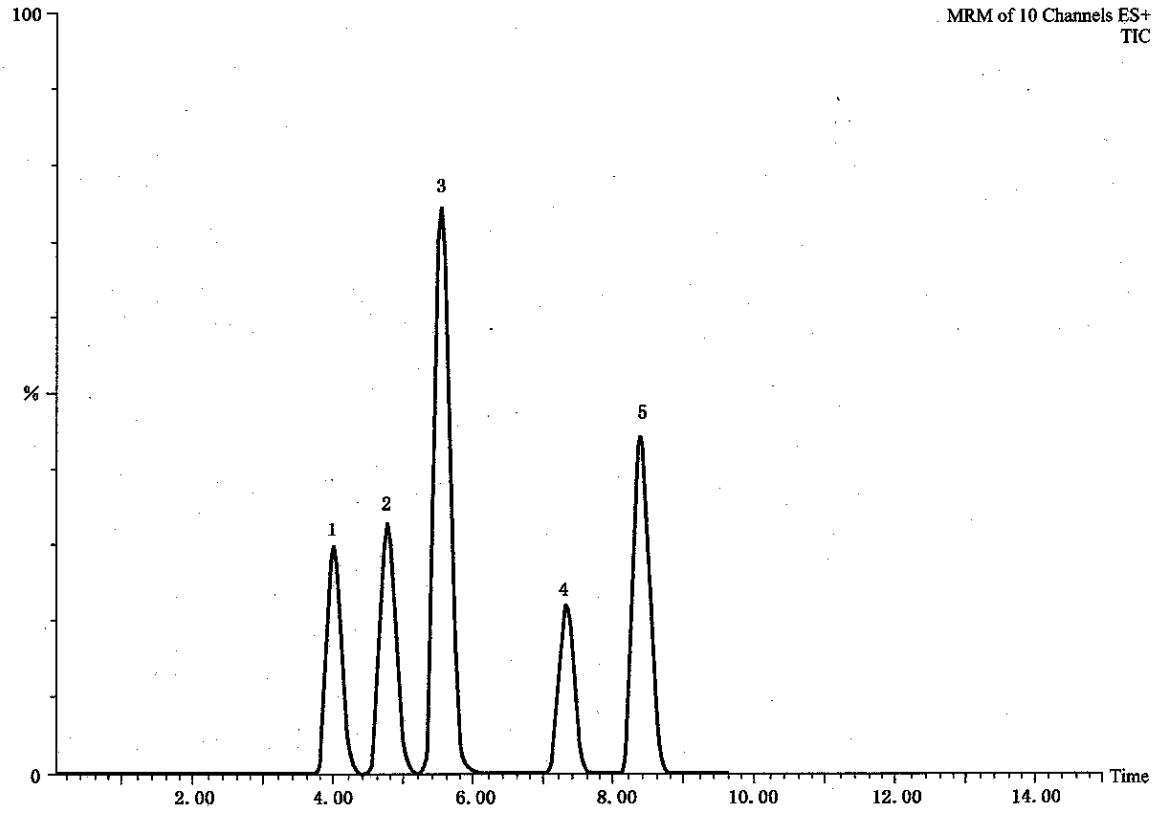
4.2 Recovery

According to the experimental data, the fortifying concentrations of each herbicide in test sample and its corresponding recoveries in wheat, barley, soybean, coleseed, rice see table 2.

Table 2 Experimental date table

Herbicide	Fortifying concentrations/(mg/kg)	Recovery
Linuron	0.01	77.3%～88.5%
	0.10	83.6%～98.6%
	1.00	88.4%～102.5%
Fluometuron	0.01	79.8%～89.5%
	0.10	75.3%～92.2%
	1.00	86.2%～98.7%
Atrazine	0.01	79.5%～86.8%
	0.10	77.8%～90.7%
	1.00	80.5%～87.4%
Propanil	0.01	79.5%～86.9%
	0.10	76.8%～90.6%
	1.00	80.5%～95.4%
Cyanazin	0.01	80.6%～89.7%
	0.10	83.9%～95.7%
	1.00	80.5%～95.0%

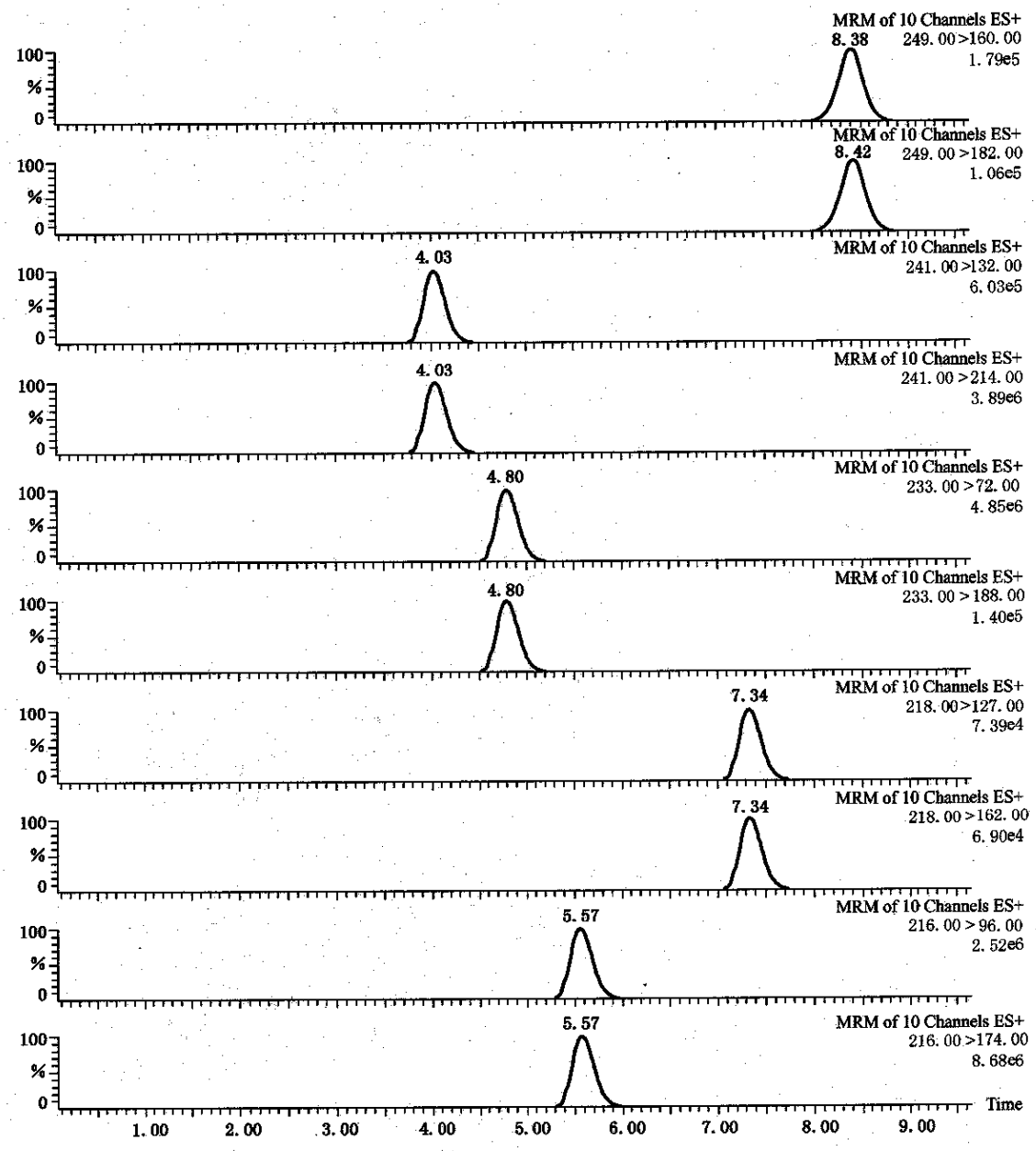
Annex A
(Informative)
HPLC-MS/MS TIC chromatogram of the standards



Note: The peak number 1,2,3,4,5 represents respectively cyanazin, fluometuron, atrazine, propanil, linuron.

Fig. A.1 TIC chromatogram of cyanazin, fluometuron, atrazine, propanil and linuron standards

Annex B
(Informative)
Chromatogram of the standards



Note: The retention times of cyanazin, fluometuron, atrazine, propanil, linuron are 4.03 min, 4.80 min, 5.57 min, 7.34 min, 8.38 min, respectively.

Fig. B.1 MRM chromatogram of cyanazin, fluometuron, atrazine, propanil and linuron standards

中华人民共和国出入境检验检疫
行 业 标 准
进出口植物性产品中氰草津、氟草隆、
莠去津、敌稗、利谷隆残留量检验方法
高效液相色谱法
SN/T 1605—2005
*
中国标准出版社出版发行
北京复兴门外三里河北街16号
邮政编码:100045
网址 www.bzcbs.com
电话:68523946 68517548
中国标准出版社秦皇岛印刷厂印刷
*
开本 880×1230 1/16 印张 1.25 字数 24 千字
2005年11月第一版 2005年11月第一次印刷
*
书号:155066·2-16449 定价 12.00 元

SN/T 1605-2005

SN/T 1605-2005