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液相色谱-串联质谱法测定饲料中三聚氰胺残留

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摘要 应用液相色谱-串联质谱法测定饲料中三聚氰胺残留。试样用 $V(\text{乙腈}):V(\text{H}_2\text{O}) = 1:1$ 溶液, 提取, 高速离心后, 供液相色谱-串联质谱仪定性定量分析。流动相为 $V(\text{乙腈}):V(\text{H}_2\text{O}) = 80:20$ 混合溶液。采用电喷雾离子源, 定性离子对为 127. 2/85. 2 和 127. 2/68. 2; 定量离子对为 127. 2/85. 2。在添加了 0. 5 ~ 10. 0 mg/kg 的三聚氰胺标准品时的回收率为 92. 6% ~ 103. 2%; 相对标准偏差 (RSD) 在 0. 8% ~ 2. 0%; 检出限为 0. 2 mg/kg。

关键词 液相色谱-串联质谱, 三聚氰胺, 饲料

1 引言

2007 年 3 月, 美国 FDA 因为部分宠物生病甚至死亡而下令回收近 100 个品牌的宠物食品后, 人们才开始高度关注三聚氰胺进入食物供应链的问题。上述宠物食品被认为是受三聚氰胺污染的小麦面筋制成。美国法律禁止在食品中添加三聚氰胺, 中国政府近期也宣布禁止在食品中添加三聚氰胺。三聚氰胺一般添加在对蛋白质含量要求比较高的产品中, 比如各种饲料蛋白粉或者称为高蛋白类食品中, 目的是提高产品的氮含量。三聚氰胺 (melamine) 又称三氨三嗪, 简称三胺, 是一种重要的氮杂环有机化工原料, 白色无味结晶粉末^[1]。这种化学品常被用于生产塑料、胶水和阻燃剂, 在部分亚洲国家, 它也被用于制造化肥。三聚氰胺是一种禁止用于宠物食品及动物饲料的化学物质, 动物食用后可以使动物发生肾衰竭并导致死亡。关于饲料中三聚氰胺残留测定方法, 目前没有相关国家标准。相关文献有 GC-MS 法、LC-UV 法^[2-5]、高效液相色谱法测定单氰胺中三聚氰胺含量^[6], 相关检测机构建立的方法也少有报道^[7,8], 新颁布的农业部标准 NY/T1372-2007 建立了 HPLC 法和 GC-MS 法。以上方法, 存在过程冗长、需要衍生、试剂用量较大及灵敏度低等缺点。本实验建立了 HPLC-MS/MS 的快速、灵敏、准确的检测方法, 并对玉米蛋白饲料、麸质饲料、98% 赖氨酸盐酸盐、65% 赖氨酸硫酸盐、苏氨酸 5 个品种 46 批饲料样品作三聚氰胺检测。本方法可完全满足饲料制品安全检测的需要。

2 实验部分

2.1 仪器、试剂与标准品

API4000 液相色谱-质谱/质谱联用仪, 配有电喷雾 (ESI) 源; 离心机 (5000 r/min); 超声提取仪。乙腈 (色谱纯), 其余试剂均为分析纯, 实验用水均为二次蒸馏水。

标准储备溶液: 准确称取 100 mg (精确到 0.1 mg) 的三聚氰胺标准品, 用 $V(\text{乙腈}):V(\text{H}_2\text{O}) = 5:5$ 溶解并定容于 100 mL 容量瓶中。配制成浓度为 1000 mg/L 的标准储备溶液。根据需要用 $V(\text{乙腈}):V(\text{H}_2\text{O}) = 1:1$ 混合溶液稀释成适用浓度的标准工作溶液。

2.2 实验步骤

称取试样约 10.0 g (精确至 0.01 g) 于 100 mL 带塞比色管中, 加入 50 mL $V(\text{乙腈}):V(\text{H}_2\text{O}) = 1:1$ 提取液, 用超声提取仪提取 30 min, 静置沉降 15 min, 取 40 mL 上层清液, 以 5000 r/min 离心 5 min, 取上层清液过 0.20 μm 有机滤膜, 待测。

2.3 仪器工作参数

2.3.1 色谱条件 色谱柱: Agilent XDBC₁₈ (150 mm \times 2.1 mm i. d, 3.5 μm); 流动相: $V(\text{乙腈}):$

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$V(\text{H}_2\text{O}) = 80:20$ 混合溶液, 流速 0.2 mL/min ; 进样量 $10 \mu\text{L}$; 柱温: $30 \text{ }^\circ\text{C}$ 。

2.3.2 质谱条件

离子源: 电喷雾 (ESI) 离子源, 正离子模式, 多反应监测 (MRM) 扫描方式; 电喷雾电压 (IS): 5500 V ; 离子源温度 (TEM): $500 \text{ }^\circ\text{C}$; 辅助气 1 压力 (GS1): 139 kPa , 辅助气 2 压力 (GS2): 206.7 kPa ; 气帘气压力 (CUR): 103.4 kPa ; 碰撞气 (CAD): 41.3 kPa ; 入口电压 (EP) 和出口电压 (CXP): 10 V 。

3 结果与讨论

3.1 液相色谱-串联质谱测定

3.1.1 液相色谱条件优化 实验了安捷伦、沃特斯等不同公司、不同型号的色谱柱, 最终选择 Agilent XDB C_{18} ($150 \text{ mm} \times 2.1 \text{ mm i. d.}$, $3.5 \mu\text{m}$), 可以满足实验要求。以不同配比的水和乙腈作为流动相, 发现不同比例的流动相对出峰时间无明显影响, 考虑到实际使用, 最终确定 $V(\text{乙腈}):V(\text{H}_2\text{O}) = 80:20$ 混合溶液为最佳流动相。

3.1.2 质谱条件的优化定性测定 根据三聚氰胺的分子结构特征, 选择电喷雾 (ESI) 离子源, 在正离子模式下对三聚氰胺进行一级质谱分析, 得到三聚氰胺的分子离子峰, 选择合适的分子离子峰进行优化, 并进行二级质谱碎片分析, 得到特征子离子信息, 根据其质谱图中的碎片离子选择了丰度相对较高和相对分子量较大的碎片, 选择合适的离子对进行电喷雾电压 (IS)、离子源温度 (TEM)、碰撞气 (CAD)、入口电压 (EP)、出口电压 (CXP)、去簇电压 (DP) 及碰撞能量 (CE) 等质谱参数优化, 使三聚氰胺的分子离子与特征碎片离子的灵敏度达到最大时定为最佳质谱条件 (见 2.2.2 节)。优化后定性离子对为 $127.2/85.2$ 、 $127.2/68.2$, 定量离子对为 $127.2/85.2$ 。检测离子对、去簇电压 (DP) 60 V 、碰撞能量 (CE) 15 V 。

本实验利用串联质谱作为检测器进行定性分析。在上述条件下, 三聚氰胺标准出峰时间为 1.31 min , 液相色谱串联质谱图和二级质谱全扫描图如图 1 和图 2 所示。

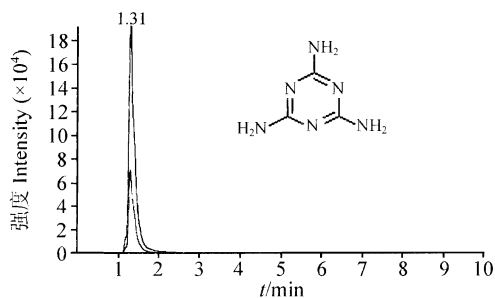


图 1 三聚氰胺标准溶液的液相色谱串联质谱图

Fig. 1 LC/MS/MS Chromatogram of melamine standard

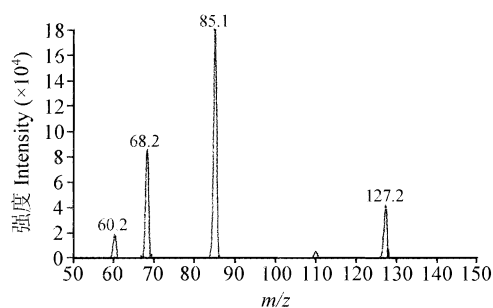


图 2 三聚氰胺二级质谱全扫描图

Fig. 2 LC/MS/MS second level scanning chromatogram of melamine standard

在扣除背景后的样品谱图中, 各定性离子的相对丰度与浓度接近的同样条件下的标准溶液谱图相比, 最大允许相对偏差不超过表 1 中的规定范围。

3.2 线性范围、检出限、回收率和精密度

配制浓度分别为 0.20 、 1.00 、 5.00 、 8.0 和 10.0 mg/L 三聚氰胺标准溶液。注入进样瓶中进行色谱测定, 按上述色谱条件进行分离, 以浓度和吸收峰面积标绘制标准曲线。以保留时间及离子对 $127.2/85.2$ 、

$127.2/68.2$ 进行定性分析, 离子对 $127.2/85.2$ 为定量离子, 外标法定量。在 $0.50 \sim 10.0 \text{ mg/L}$ 质量浓度范围内各物质的峰面积 (Y) 与浓度 (x) 具有良好的线性关系。线性回归方程为 $Y = 206851x + 10478.6$, 相关系数 $r = 0.9998$ 。

表 1 定性确证时相对离子丰度的最大允许相对偏差

Table 1 Most allowed warp of relatively ion in determine corroboration

相对离子丰度 Relative ion abundance	>50%	20% ~ 50%	10% ~ 20%	≤10%
允许相对偏差 Relative deviation	±20%	±25%	±30%	±50%

对10个近似空白的水样进行测定,得出响应值标准差,按空白值3倍标准差计算检出限,将峰面积换算成被测物浓度,三聚氰胺的检出限为0.2 mg/kg。

进行回收实验时,三聚氰胺的标准品应采用样品基质溶液进行溶解及配制。三聚氰胺的标准添加量为1.00 mg/kg,方法回收率为92.6%~103.2%。

3.3 实际样品的测定

取实验室制备的含有三聚氰胺的饲料样品,按处理方法进行提取测定,连续测定6次,以实际测定浓度的相对标准偏差表征方法的精密度。三聚氰胺峰面积的相对标准偏差(RSD)在0.8%~2.0%之间。三聚氰胺主要用于高蛋白宠物饲料中,本方法选取了玉米蛋白饲料、麸质饲料、98%赖氨酸盐酸盐、65%赖氨酸硫酸盐及苏氨酸做为检测品种。这5种饲料是我国主要的饲料出口品种。采用本方法对近期(2007年3月起)出口饲料样品进行检测,总计检测了包括玉米蛋白饲料、麸质饲料、98%赖氨酸盐酸盐、65%赖氨酸硫酸盐、苏氨酸等5个品种46批饲料样品,结果仅有1个样品检测出三聚氰胺,三聚氰胺残留量为9.0 mg/kg。

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Determination of Melamine Residues in Feed by Liquid Chromatography Tandem Mass Spectrometry

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Abstract A sensitive method was developed for the determination of melamine residues in feed by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Test sample is extracted with water-acetonitrile (1:1, V/V). The extract is centrifuged and the supernatant is determined and confirmed by LC/MS/MS. Mobile phase in LC is water-acetonitrile (80:20, V/V). Electro-spray ionization source is applied. The determination ion pairs are 127.2/85.2 and 127.2/68.2, and the confirmation ion pairs are 127.2/85.2. While the spiked contents of melamine standard are 0.5 - 10.0 mg/kg, the recoveries are in the range of 92.6% - 103.2%, the RSDs are in 0.8% - 2.0%. The limit of determination is 0.2 mg/kg.

Keywords Liquid chromatography-electrospray tandem mass spectrometry, melamine residues, feed

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