

异丙甲草胺农药残留吸收光谱检测方法研究

王晓燕^{1,2}, 季仁东², 陈仁文^{1*}

1. 南京航空航天大学航空宇航学院, 江苏 南京 210016

2. 淮阴工学院电子信息工程学院, 江苏 淮安 223003

摘要 除草剂可以快速、有效地进行除草, 已被广泛应用, 但是在除草剂使用同时也会对周围环境和农作物带来一定程度的污染, 例如农业生产过程中经常发现由于除草剂使用不当而使果树中毒的现象。异丙甲草胺是一种酰胺类选择性除草剂, 被广泛应用于旱地作物、蔬菜和果园、苗圃。根据相关文献报道, 基于气相色谱法、气相色谱-质谱联用法和固相萃取等方法可以实现异丙甲草胺残留检测, 而基于吸收光谱法对异丙甲草胺的分析未见相关文献报道, 提出直接利用吸收光谱及其导数光谱分析法实现异丙甲草胺农药及其在苹果汁中的农药残留检测。首先利用分光光度计对不同浓度异丙甲草胺药液进行吸收光谱实验研究, 发现在 266 nm 处有明显吸收光谱特征峰。对农药吸收光谱进行拟合分析, 得到异丙甲草胺药液浓度和吸光度之间预测模型函数方程, 函数方程为 $y=2.147\ 09x+0.031\ 98$, 相关系数为 0.998 5。然后利用分光光度计对苹果汁-异丙甲草胺混合溶液进行吸收光谱实验研究, 相对于纯苹果汁吸收光谱, 在混合溶液吸收光谱中发现 266 nm 处为异丙甲草胺所对应的特征峰。对苹果汁中药物浓度和吸光度进行建模, 模型函数为: $y=0.704\ 9+0.826\ 8x$, 其相关系数为 0.991 1。可以看出, 当苹果汁中异丙甲草胺残留量很低时, 其农药吸收光谱特征峰并不明显。为进一步提高检测效果, 对混合溶液吸收光谱进行一阶导数处理, 得到其一阶导数吸收光谱。与苹果汁吸收导数光谱相比较, 苹果汁-农药混合溶液导数光谱有两个明显特征光谱峰, 分别位于 269 和 276 nm 处。进一步分析苹果汁-异丙甲草胺混合溶液的导数吸收光谱峰值与农药含量之间的关系, 对异丙甲草胺含量与导数光谱吸光度进行函数拟合。其中 269 nm 对应预测模型函数关系式: $y=0.005\ 3-0.090\ 6x$, 相关系数 $r=0.992\ 5$; 276 nm 对应预测模型函数关系式为 $y=-0.000\ 769-0.302\ 8x$, 相关系数 $r=0.990\ 6$ 。为验证由吸收光谱和其一阶导数光谱所得苹果汁中农药残留预测模型的准确性, 另外配置五种浓度苹果汁-异丙甲草胺混合溶液。然后在同等条件下对其进行吸收光谱实验, 将 266, 269 和 276 nm 处的吸光度分别代入对应模型函数可求得浓度预测值, 结合已知浓度值可计算其平均回收率, 其中吸收光谱 266 nm 对应平均回收率为 104.68%, 导数光谱 269 nm 对应平均回收率为 104.59%, 276 nm 对应平均回收率为 105.18%。对苹果汁中异丙甲草胺检测模型进行分析, 计算得到检出限(LOD)和定量限(LOQ)参数值, 其中原始吸收光谱对应 LOD 和 LOQ 分别为 0.014 8 和 0.049 2 $\text{mg} \cdot \text{mL}^{-1}$, 一阶导数光谱对应 LOD 和 LOQ 最小值分别为 0.001 5 和 0.004 9 $\text{mg} \cdot \text{mL}^{-1}$ 。研究结果表明, 采用吸收光谱方法对苹果汁中异丙甲草胺进行直接检测与分析是快速和可行有效的, 而且对吸收光谱进行导数运算处理后, 检测效果更优。

关键词 农药残留; 异丙甲草胺; 吸收光谱; 导数光谱

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作者简介: 王晓燕, 女, 1981年生, 南京航空航天大学博士研究生 e-mail: wxygxy@163.com

* 通讯联系人 e-mail: rwchen@nuaa.edu.cn

引 言

除草剂可以快速、有效地除草,已被世界各国广泛应用,但是在除草剂使用同时也会对周围环境和农作物带来一定程度的污染^[1-2]。农业生产过程中经常发现由于除草剂使用不当而使果树中毒的现象,包括由于使用浓度过高造成药害,或者有些果园本身并未使用除草剂,而是周边大田作物喷洒除草剂漂移而产生一定的危害^[3]。

异丙甲草胺是一种酰胺类选择性除草剂,其分子式为 $C_{15}H_{22}ClNO_2$ 。该农药被广泛应用于旱地作物、蔬菜作物和果园、苗圃,可防除牛筋草、马唐等一年生禾本科杂草以及苋菜等阔叶杂草和碎米莎草、油莎草。作用机制主要抑制是发芽种子的蛋白质合成,其次抑制胆碱渗入磷脂,干扰卵磷脂形成^[4]。

异丙甲草胺在农业生产中大量使用,其农药残留必然会给消费者带来潜在的危险。为减少农药环境污染,找到一种快速有效的检测方法显得尤为重要。根据相关文献报道,已开展的异丙甲草胺药物研究包括基于气相色谱法(GC)^[5]、气相色谱-质谱(GC-MS)联用法^[6-8]、固相萃取(SPE)^[9]等方法进行残留检测,基于光谱法研究异丙甲草胺的生物活性^[10],以及异丙甲草胺残留降解动态研究^[11-13]。紫外可见吸收光谱法具有操作简单、分析快速等优点,被广泛应用于物质成分的定性或定量分析^[14-15]。本工作直接利用吸收光谱分析方法对异丙甲草胺农药及其在苹果汁中农药残留进行检测与研究,研究结果具有一定应用参考价值。

1 实验部分

1.1 样品

纯度 100% 苹果汁和异丙甲草胺农药。

1.2 仪器

UV6300 紫外-可见分光光度计用于记录样品的吸收光谱,设置仪器技术指标:波长扫描范围:190~800 nm;光谱分辨率 1 nm。

1.3 方法

将异丙甲草胺农药配成不同浓度比的标准药液。用微量量筒量取各种浓度比药液 3 mL 放入比色皿,由分光光度计获取吸收光谱。将纯苹果汁和纯净水按照一定体积比进行稀释,用分光光度计检测混合溶液的吸收光谱。利用比色皿取稀释后苹果汁 2.5 mL,然后利用微量量筒抽取浓度为 $0.6 \text{ mg} \cdot \text{mL}^{-1}$ 的异丙甲草胺标准药液逐量加入到苹果汁中,每次加入后进行充分搅拌,使药液和果汁混合均匀,检测果汁-异丙甲草胺混合体系的吸收光谱。

2 结果与讨论

2.1 异丙甲草胺吸收光谱

对所配置的异丙甲草胺标准药液利用分光光度计检测其吸收光谱,其结果如图 1(a)所示,横坐标表示光的波长,纵

坐标表示吸光度。图中从 1—5 分别对应 $0.023, 0.053, 0.12, 0.27$ 和 $0.6 \text{ mg} \cdot \text{mL}^{-1}$ 五种浓度比的异丙甲草胺药液。可以看出在 266 nm 出现吸收光谱峰,而且随着浓度值的增加,其峰值相应提高。因此,可以利用 266 nm 作为异丙甲草胺药液吸收光谱的特征峰。为分析异丙甲草胺浓度和吸光强度之间的相互关系,对 266 nm 处不同浓度异丙甲草胺药液浓度值与吸光度之间进行函数拟合,结果如图 1(b)所示,结果表明在所选浓度范围内浓度值与吸光度具有很好的线性关系,相关系数为 0.998 5,线性函数为 $y=2.147 09x+0.031 98$ 。

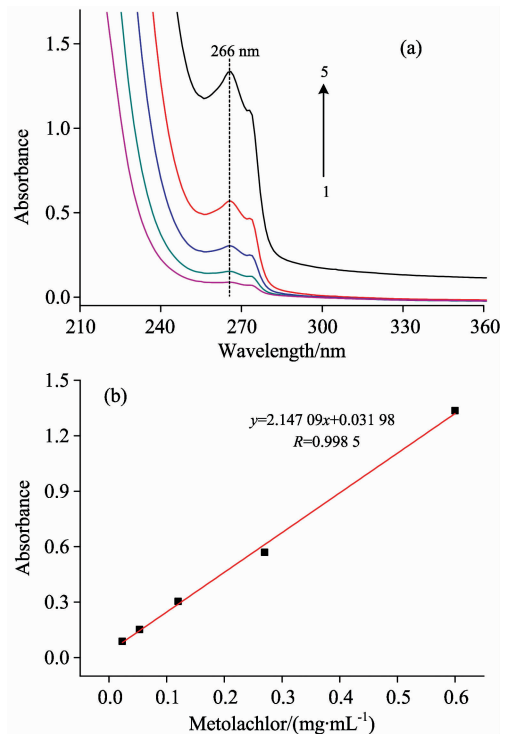


图 1 异丙甲草胺吸收光谱及检测函数建模

(a): 1—5 表示异丙甲草胺溶液浓度值分别为 $0.023, 0.053, 0.12, 0.27$ 和 $0.6 \text{ mg} \cdot \text{mL}^{-1}$; (b): 异丙甲草胺药液在 266 nm 处浓度值与吸光度之间的函数关系

Fig. 1 The absorption spectrum of metolachlor and detection function modeling

The metolachlor solution is corresponding to $0.023, 0.053, 0.12, 0.27$ and $0.6 \text{ mg} \cdot \text{mL}^{-1}$ from curve 1 to 5 in (a), and the relationship between the concentration of metolachlor and the absorbance intensity at 266 nm was shown in (b)

2.2 果汁和异丙甲草胺混合溶液吸收光谱

利用微量量筒取稀释后苹果汁 2.5 mL 于 1cm 比色皿中,再将 $0.6 \text{ mg} \cdot \text{mL}^{-1}$ 的异丙甲草胺标准溶液逐量逐次添加到苹果汁中,利用分光光度计得到混合溶液吸收光谱,结果如图 2(a)所示,图中箭头指向从 1 到 6 表示对应苹果汁中农药浓度分别约为: $0, 0.023 1, 0.064 3, 0.100 0, 0.131 3$ 和 $0.158 8 \text{ mg} \cdot \text{mL}^{-1}$ 。可发现混合溶液吸收光谱中 266 nm 处有吸收光谱峰,随着药物浓度值增加,其吸光度都相应增加,根据 2.1 节分析结果可知 266 nm 为异丙甲草胺药物特

征峰。进一步分析苹果汁中药物浓度和吸光度之间的模型函数, 对其进行线性函数拟合, 结果如 2(b) 所示, 其相关系数为 0.991 1, 模型函数为: $y=0.704\ 9+0.826\ 8x$ 。

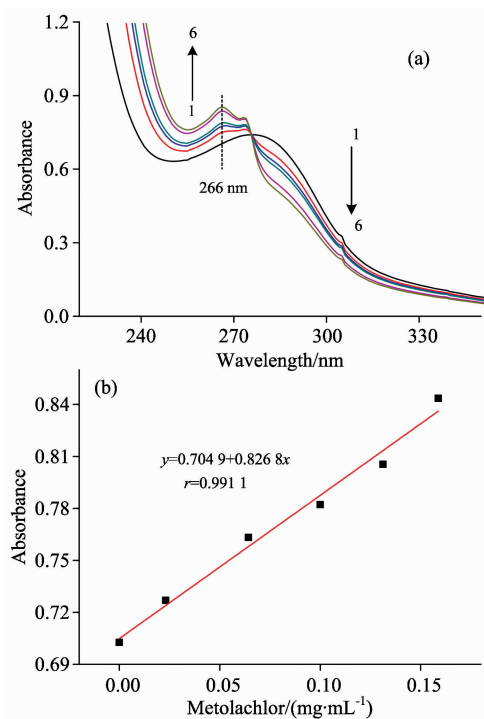


图 2 苹果汁-异丙甲草胺混合溶液吸收光谱及检测函数建模

(a) 1—6 表示苹果汁中异丙甲草胺溶液浓度值分别为 0, 0.023 1, 0.064 3, 0.100 0, 0.131 3 和 0.158 8 $\text{mg} \cdot \text{mL}^{-1}$; (b): 浓度值与 266 nm 处吸光度之间的函数关系

Fig. 2 The absorption spectrum of mixed solution of apple juice and metolachlor detection function modeling

The metolachlor solution is corresponding to 0, 0.023 1, 0.064 3, 0.100 0, 0.131 3, 0.158 8 $\text{mg} \cdot \text{mL}^{-1}$ from curve 1 to 6 in apple juice in (a), and the relationship between concentration of metolachlor and absorbance intensity at 266 nm was shown in (b)

从图 2(a) 中可以看出, 苹果汁中异丙甲草胺残留量很低时, 其农药吸收光谱特征峰并不明显, 为更进一步提高检测效果, 对混合溶液吸收光谱进行一阶导数处理, 导数吸收光谱如图 3 所示。图中从 1 到 6 分别对应苹果汁中农药浓度分别约为 0, 0.023 1, 0.064 3, 0.100 0, 0.131 3 和 0.158 8 $\text{mg} \cdot \text{mL}^{-1}$ 。与苹果汁吸收导数光谱相比较, 苹果汁-农药混合溶液导数光谱有两个明显特征光谱峰, 分别位于 269 和 276 nm 处。而与原始吸收光谱相比较, 采用一阶导数光谱更有利于发现苹果汁中异丙甲草胺农药残留。

为进一步分析苹果汁-异丙甲草胺混合溶液吸收导数光谱峰值随农药含量变化的关系, 对异丙甲草胺农药含量与导数光谱吸光度进行线性拟合, 结果如图 4 所示。其中 269 nm 对应预测模型函数关系式: $y=0.005\ 3-0.090\ 6x$, 相关系数 $r=0.992\ 5$; 276 nm 对应预测模型函数关系式为 $y=-0.000\ 769-0.302\ 8x$, 相关系数 $r=0.990\ 6$ 。

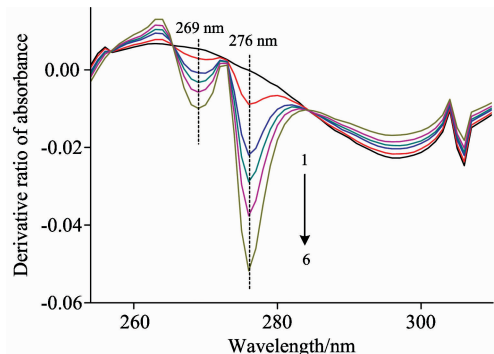


图 3 苹果汁-异丙甲草胺混合溶液吸收光谱一阶导数光谱 1—6 表示苹果汁中异丙甲草胺溶液浓度值分别为 0, 0.023 1, 0.064 3, 0.100 0, 0.131 3 和 0.158 8 $\text{mg} \cdot \text{mL}^{-1}$

Fig. 3 The first derivative spectrum of mixed solution of apple juice and metolachlor

The metolachlor solution is corresponding to 0, 0.023 1, 0.064 3, 0.100 0, 0.131 3 and 0.158 8 $\text{mg} \cdot \text{mL}^{-1}$ from curve 1 to 6 in apple juice

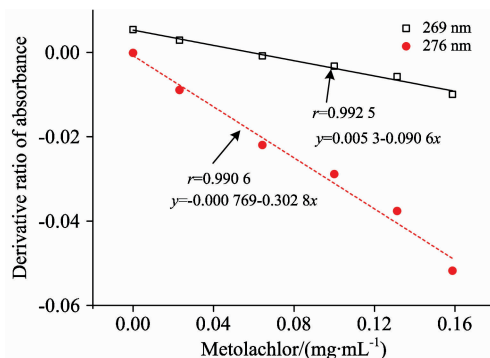


图 4 苹果汁-异丙甲草胺混合溶液吸收光谱一阶导数光谱检测函数建模 (269 和 276 nm)

Fig. 4 The relationship between metolachlor content in apple juice and derivative ratio of absorbance (269 and 276 nm)

2.3 果汁中异丙甲草胺检测模型函数分析

为验证所得苹果汁中异丙甲草胺残留预测模型准确性, 另外配置五种浓度苹果汁-异丙甲草胺混合溶液, 浓度值分别为 0.044 4, 0.082 8, 0.116 1, 0.145 5 和 0.171 4 $\text{mg} \cdot \text{mL}^{-1}$ 。然后在同等条件下对其进行吸收光谱检测, 将 266, 269 和 276 nm 处相应吸光度代入对应模型函数可求得预测量, 然后结合已知浓度值得到其平均回收率参数。另外对所建立苹果汁中异丙甲草胺检测模型函数进行分析计算, 根据 $\text{LOD}=3(\delta/S)$, $\text{LOQ}=10(\delta/S)$ 的计算方法可分别得到检出限 (LOD)、定量限 (LOQ) 等参数值, 结果如表 1 所示。

表 1 中数据显示基于原始吸收光谱和一阶导数吸收光谱所建立的异丙甲草胺在苹果汁中检测模型函数的相关系数比较接近, 而且都超过了 0.99, 吸光度和农药浓度具有很好的线性相关性。同样根据所配置的五种苹果汁-农药混合溶液数据进行分析, 两种光谱模型函数都具有很好的回收率。从

检出限和定量限两个参数来看,基于一阶导数吸收光谱所构建药物检测模型函数明显优于原始吸收光谱,例如原始吸收光谱对应 LOD 和 LOQ 分别为 0.014 8 和 0.049 2 mg · mL⁻¹,一阶导数光谱对应 LOD 和 LOQ 最小值分别为

0.001 5 和 0.004 9 mg · mL⁻¹。因此从光谱特征峰与检测模型函数参数两个角度都表明,采用导数吸收光谱更有利于检测异丙甲草胺农药残留。

表 1 苹果汁中异丙甲草胺含量预测函数相关参数

Table 1 The evaluation parameter values of predicted function about metolachlor in apple juices

Parameter	Original absorption spectrum method	First derivative of the ratio spectrum method	
Wavelength/nm	266	269	276
Correlation coefficient(<i>R</i>)	0.991 1	0.992 5	0.990 9
LOD/(mg · mL ⁻¹)	0.014 8	0.002 4	0.001 5
LOQ/(mg · mL ⁻¹)	0.049 2	0.007 8	0.004 9
The mean recovery/%	104.68	104.59	105.18

3 结 论

(1)对于不同浓度的异丙甲草胺药液紫外可见吸收光谱在 266 nm 都有着稳定的特征峰,而且随着农药浓度值的增加,其吸光度相应增加。对异丙甲草胺药液进行浓度值与 266 nm 处吸光度线性拟合,发现具有很好的线性关系,相关系数超过 0.99。

(2)向 100%苹果汁中逐量添加 0.6 mg · mL⁻¹异丙甲草胺标准溶液,得到苹果汁-异丙甲草胺混合体系吸收光谱,并进行一阶导数数据处理,混合溶液导数光谱相对苹果汁导数光谱更具有显著特征峰。通过对原始光谱和导数光谱对应的

特征峰值与异丙甲草胺药液浓度进行函数拟合,得到基于吸收光谱和一阶导数吸收光谱两种情况下苹果汁中异丙甲草胺农药含量与吸光度之间的预测函数模型及相关系数,相关系数都超过 0.99。

(3)根据所得预测模型函数,对苹果汁中异丙甲草胺含量进行验证分析,得到平均回收率以及检测限等参数。比较发现,所建模型函数都具有很好的回收率,同时从检出限参数来看,基于一阶导数光谱异丙甲草胺残留预测模型要优于原始吸收光谱。

采用吸收光谱方法对苹果汁中异丙甲草胺农药进行直接检测与分析是快速和可行有效的,而且对吸收光谱进行导数运算处理后,检测效果更佳。

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Research on Detection of Metolachlor Pesticide Residue by Absorption Spectroscopy

WANG Xiao-yan^{1, 2}, JI Ren-dong², CHEN Ren-wen^{1*}

1. College of Aerospace Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 210016, China

2. Faculty of Electronic Information Engineering, Huaiyin Institute of Technology, Huai'an 223003, China

Abstract Herbicides have been widely used because of their rapid and effective weeding. But it will also cause a certain degree of pollution to the environment and the crops, and it is often found that the misuse of herbicides causes the fruit trees to be poisoned in the process of agricultural production. Metolachlor is a selective amide herbicide which is widely used in upland crops, vegetable crops, orchard and nursery. According to the relevant literature, the methods of Metolachlor residue detection mainly include Gas chromatography (GC), Gas chromatograph-mass spectrometry (GC-MS), Solid phase extraction (SPE) and so on. The analysis of metolachlor residues based on absorption spectroscopy has not been reported in literature. This paper presented the absorption spectroscopy and its derivative spectrometry to detect the metolachlor pesticide residues in the apple juice. First, the absorption spectrum of different concentration of metolachlor were recorded by spectrophotometer, and it was found that there was a distinct absorption spectrum peak at 266 nm. The relationship between the pesticide concentration and absorbance was obtained by fitting analysis on the absorption spectrum of metolachlor, and the function equation was deduced as $y = 2.147\ 09x + 0.031\ 98$, and the correlation coefficient was 0.998 5. Second, the absorption spectrum of mixed solution of apple juice and metolachlor were studied by spectrophotometer. Compared with the absorption spectrum of apple juice, the metolachlor characteristic peak at 266 nm was also found in the absorption spectrum of mixed solution. The model function between absorbance and metolachlor concentration in apple juice was further obtained as follows: $y = 0.704\ 9 + 0.826\ 8x$, its correlation coefficient was 0.991 1. It can be seen that when the residual amount of metolachlor in apple juice was very low, the absorption spectrum characteristic peak of the pesticide was not obvious. Third, in order to further improve the detection effect, the first derivative processing of the absorption spectrum was carried out, and the first order derivative absorption spectrum of the mixed solution were then obtained. Compared with the derivative absorption spectrum of apple juice, the derivative absorption spectrum of mixed solution of apple juice and metolachlor pesticide had two distinct spectral peaks, which were located at 269 and 276 nm, respectively. In order to further analyze the relationship between the metolachlor content and the peak value of the derivative absorption spectrum of mixed solution, the metolachlor content and the absorbance of derivative spectrum were linearly fitted, and the corresponding prediction model in 269 nm was deduced as $y = 0.005\ 3 - 0.090\ 6x$, and the correlation coefficient was $r = 0.992\ 5$. The prediction model corresponding to 276 nm was deduced as $y = -0.000\ 769 - 0.302\ 8x$, and the correlation coefficient was $r = 0.990\ 6$. At last, in order to verify the accuracy of the prediction model obtained from the absorption spectrum and its first derivative spectrum, five different concentrations of the mixed solution of metolachlor and apple juice were configured and tested under the same experiment condition. The absorbance value at 266, 269 and 276 nm were substituted into the model function respectively, and the predictive value of the metolachlor concentration can be obtained, the average recovery rate can be further calculated according to the actual metolachlor concentration. The calculation results were as follows: the average recovery rate of the absorption spectrum at 266 nm was 104.68%, and the average recovery rate of the derivative spectrum at 269 nm was 104.59%, and it was 105.18% at the absorption peak of 276 nm. The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated by analyzing the detection model of metolachlor in apple juice. The LOD and LOQ of the original absorption spectrum were $0.014\ 8\ \text{mg} \cdot \text{mL}^{-1}$ and $0.049\ 2\ \text{mg} \cdot \text{mL}^{-1}$, respectively. And the minimum values of LOD and LOQ corresponding to the first derivative absorption spectra were $0.001\ 5\ \text{mg} \cdot \text{mL}^{-1}$ and $0.004\ 9\ \text{mg} \cdot \text{mL}^{-1}$, respectively. The results showed that absorption spectroscopy was fast and feasible for the detection and analysis of metolachlor residue in apple juice, and the detection effect was better after the derivative operation of absorption spectrum.

Keywords Pesticide residue; Metolachlor; Absorption spectrum; Derivative spectrum

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* Corresponding author