Asian Journal of Chemistry Vol. 17, No. 3 (2005), 1617–1624

Quality Control Analysis of Plant Growth Regulator Forchlorfenuron by Reversed Phase High Performance Liquid Chromatography and Liquid Chromatography-Mass Spectrometry

HONGZHEN LIAN*, DANNI LI and WEI ZHU
The Key Laboratory of Life Analytical Chemistry of Ministry of Education
Center of Materials Analysis and Department of Chemistry
Nanjing University, Nanjing 210093, China
E-mail: hzlian@nju.edu.cn
Fax: +(86)(25)83325180 Tel: +(86)(25)83686075

A reliable reversed-phase high performance liquid chromatographic method has been developed for simultaneous determination of plant growth regulator forchlorfenuron, as well as its related impurities 4-amino-2-chloropyridine and N,N′-diphenylurea. Separation was achieved on a Kromasil C_{18} column by using methanol: water (60 : 40 v/v) as the mobile phase and detection was operated by UV adsorption at a wavelength of 254 nm. The identification for all interested substances was performed by photodiode-array detection and mass spectrometry coupled to liquid chromatography. The results indicated that the relative standard divisions for determinations of forchlorfenuron, 4-amino-2-chloropyridine and N,N′-diphenylurea were 0.18–0.29, 0.54–0.78 and 0.33–1.50%, with the recoveries of 99.59–101.99, 98.77–104.06 and 97.98–103.49%, respectively. This method is found to be superior to previous gas or high performance liquid chromatographic methods and to be an attractive choice for the quality control of forchlorfenuron.

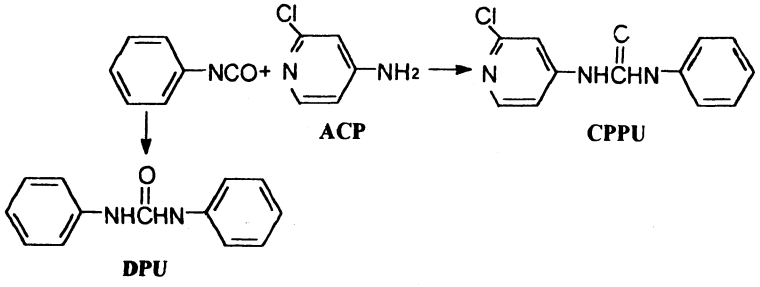
Key Words: Forchlorfenuron, 4-Amino-2-chloropyridine, N,N′-Diphenylurea, Quality control analysis, High performance liquid chromatography, UV detection, Mass spectrometry.

INTRODUCTION

Forchlorfenuron (N-(2-chloro-4-pyridinyl)-N′-phenylurea, CPPU), a kind of urea cytokinin, can induce the anthocyanin accumulation as well as the carbohydrate accumulation in some plants such as radish, tomato, apple, berry, grape, etc. Therefore, it is used as a growth regulator. It possesses higher cytokinin activity than other available adenine-type cytokinins and is considered to be an ideal substitute for zeatin for agricultural use in view of its simplicity and low cost in chemical synthesis[1, 2]. Recently, there were some methods including gas chromatography (GC)[3, 4] and reversed-phase high performance liquid chromatography (RP-HPLC)[5, 6] developed to analyze CPPU. However, no method was reported to simultaneously detect CPPU and coexisting impurities. It is needed to establish the method for the analysis of all components in a pesticide including such a plant growth regulator in order to be registered and traded in many countries.

CPPU is usually obtained by the following reaction[1, 2]. There may be trace reactant 4-amino-2-chloropyridine (ACP) and by-product N,N′-diphenylurea

(DPU) in the final products. Therefore, not only the main content of CPPU has to be determined, but also the contents of these related substances have to be controlled during the production process. In this paper, the results from the qualitative and quantitative analysis by RP-HPLC with UV detection combined with liquid chromatography-mass spectrometry (LC-MS) are demonstrated.



EXPERIMENTAL

The HPLC system for quantitative analysis consisted of Waters Alliance 2695 Separations Module equipped with a vacuum degasser, a quaternary pump, an autosampler, and a 996 UV-Vis photodiode-array detector (PDA) (Waters, Milford, MA, USA). The separation was controlled and the chromatograms and spectra were recorded by a Waters Millinium^{32} chromatography manager system. The LC-MS analysis was performed using the Mariner^{TM} 5140 time-of-flight (TOF) mass spectrometer with Mariner^{TM} 4.0 workstation (Applied Biosystem, Foster, CA, USA), coupled to a Waters Alliance 2690 Separations Module with a 996 PDA detector system (Waters).

Reference substances (RS) (purity > 99%) of N-(2-chloro-4-pyridinyl)-N′-phenylurea, 4-amino-2-chloropyridine and N,N′-diphenylurea were provided by Jiangsu Jintan Maosheng Additive Reagent Factory, Changzhou, PRC. Their structures were confirmed by using FT-IR, MS and ^{1}H NMR. Methanol was HPLC grade (Hanbang Sci. & Tech Co. Ltd., Jiangsu, Huaian, PRC). Water used for all solutions, dilution and mobile phase was distilled twice from quartz.

Operation Conditions: The column was a Kromasil C_{18}, 150 × 4.0 mm I.D., packed with 5 μm particle (Hanbang). The mobile phase was methanol : water (60 : 40 v/v). The separation was carried out by isocratic elution with a flow rate of 1.0 mL/min, and the column temperature was maintained constant at 30°C. The injection volume was 10 μL throughout.

MS detection was performed with an electrospray ionization (ESI) interface and operated in the positive-ionization mode. Mass detector : spray tip potential 5190 V; nozzle potential 140 V; detector voltage 2375 V; Nozzle temperature 140°C; scanning mass range: 100–1000.

System suitability test: A mixture solution of CPPU, ACP and DPU was used to optimize the peak resolution. About 50 mg of ACP and 50 mg of DPU were weighed into a 100 mL volumetric flask, respectively. Added mobile phase, dissolved and diluted to volume. About 100 mg of CPPU was weighed into a 100-mL volumetric flask. Added 1.0 mL of ACP solution and 0.5 mL of DPU solution. Added mobile phase to volume and mixed. Injected 10 μL of the system suitability solution on to the analytical column.

Identification of impurities: A sample solution was prepared by weighing about 100 mg of a CPPU commercial sample into a 100 mL volumetric flask and adding mobile phase to make up to the mark. 10 μL of the solution prepared above was injected on to the column. The PDA detector was used to collect the UV spectra, with the wavelength ranging from 210 to 400 nm, of all components corresponding to the peaks in the chromatogram. Moreover, LC-MS was employed to record the mass spectra of these components.

Quantitative determination of CPPU and impurities in samples: The sample solution, for analyses of related impurities, was prepared by weighing accurately about 100 mg of commercial sample into a 100 mL volumetric flask and adding mobile phase to the mark. 10 μL of the sample solution was injected on to the column. For CPPU determination, 2.0 mL of the obtained sample solution was transferred into a 100 mL volumetric flask, and then mobile phase was added to volume. 10 μL of the diluted sample solution was injected on to the column.

RESULTS AND DISCUSSION

Conditions for separation: A HPLC chromatogram generated at 254 nm demonstrating the separation of an RS mixture solution of CPPU, ACP and DPU (prepared as in system suitability test) is shown in Fig. 1-a. As can be seen, the retention order of peaks is ACP, DPU and CPPU with retention times of 2.1, 5.9 and 6.9 min respectively. The resolution between the peaks of DPU and CPPU was more than 1.5. By comparing with separation phenomenon under various methanol proportions in mobile phase, it indicates that three components can be baseline separated under methanol : water (60 : 40 v/v) condition (shown in Fig. 1).

PDA and mass spectra: Three peaks are observed during a chromatographic run for a sample solution (prepared as in identification of inpurities), with the

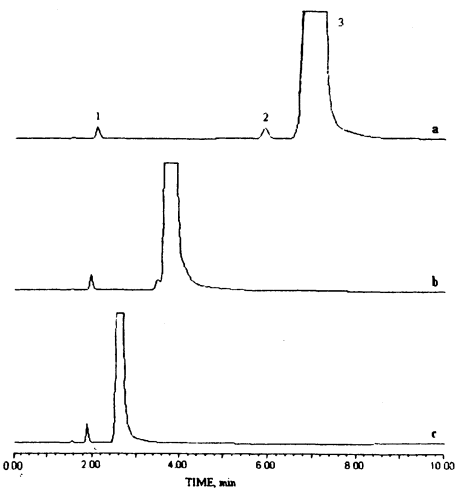


Fig. 1. Chromatogram of RS mixture solution containing ACP, DPU and CPPU under different mobile phase proportions: (a) methanol : water (60 : 40 v/v); (b) methanol : water (70 : 30 v/v); (c) methanol : water (80 : 20 v/v). Wavelength used for UV detection 254 nm. Other HPLC conditions as in "Operation Conditions". Peaks: 1. ACP; 2. DPU; 3. CPPU

retention times of 2.1, 5.9 and 6.9 min. These peaks are considered ACP, DPU and CPPU since their UV spectra match those of ACP, DPU and CPPU (reference substances) as shown in Fig. 2.

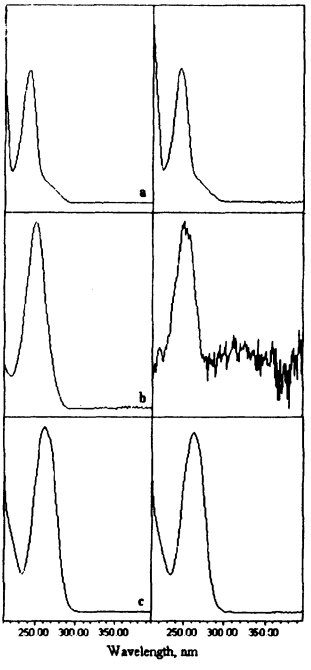


Fig. 2. PDA spectra of ACP, DPU and CPPU. The left hand: in RS mixture solution; the right hand: in sample solution. (a) ACP; (b) DPU; (c) DPPU. HPLC conditions as in Operation Conditions

LC-MS was employed to obtain further structure information. The mass to charge ratios (m/z) of the protonated molecule $(M + H)^+$ corresponding to these peaks in the sample solution are 129.0221 (131.0198, from ^{37}Cl isotope), 213.1030 and 248.0575 (250.0556, from ^{37}Cl isotope), respectively, indicating monoisotopic molecular weights of 128 (130), 212 and 247 (249) (Fig. 3).

Furthermore, the mass spectra are identical with those of ACP, DPU and CPPU RS (not shown), which verified the above consideration.

Linear range and detection limit: The quantitation was based on the calibration curve by peak area measurement with UV detection at 254 nm. This detection wavelength would insure determination sensitivity for all components. Linear regression analyses of the relationship between peak areas *vs.* amounts of standards were carried out within the range 0.0001–0.4 mg/mL for CPPU, 0.0001–0.06 mg/mL for ACP and 0.00005–0.03 mg/mL for DPU in a 10 μL injection volume. The equations were A_{CPPU} (area) = –13313.94609 + 6.88958E7 C_{CPPU}, A_{ACP} (area) = –445.90292 + 1.39318E7 C_{ACP} and A_{DPU} (area) = 75.39214 + 4.32183E7 C_{DPU}, with correlation coefficients of 0.99944, 0.99911 and 0.99970,

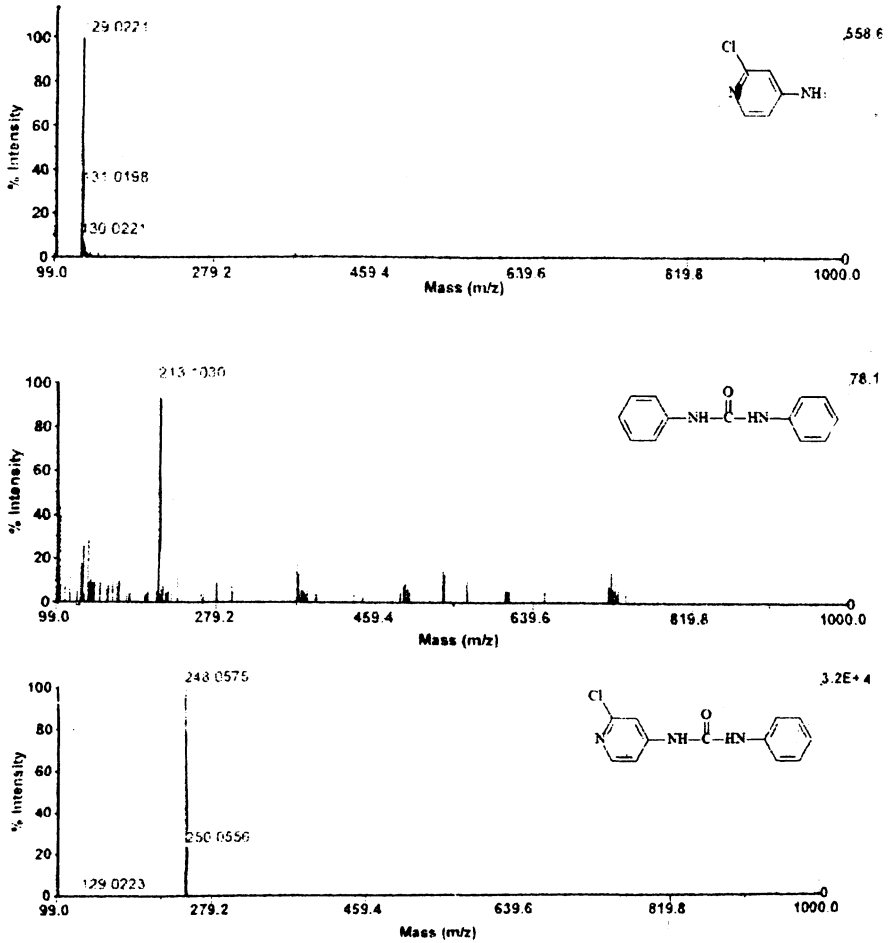


Fig. 3. Mass spectra of ACP, DPU and CPPU in sample solution. (LC-MS conditions as in Operation Conditions)

respectively. The limits of detection (S/N = 3) are 0.00001 mg/mL, 0.000005 mg/mL and 0.00001 mg/mL, respectively, for CPPU, ACP and DPU.

Results for sample determination: Five samples of CPPU commercial products were treated as in 2.6 and analyzed under the optimum HPLC conditions (Fig. 4 and Fig. 5). Considering determination of all substances in the sample, the low sample concentration was optioned for the CPPU determination, whereas the high was used to determine impurities. If sample concentration was too high for the CPPU peak to fall into the linear range, the quantitation was incorrect. But too low concentration would cause impurities to be below detection limits. Experimental precision looked good after diluting 50 times. The results are summarized in Table-1. In order to estimate the efficiency of the recovery, the five samples were spiked with the three target analytes. Table-2 shows the concentrations added of CPPU, ACP and DPU and the percentage recoveries.

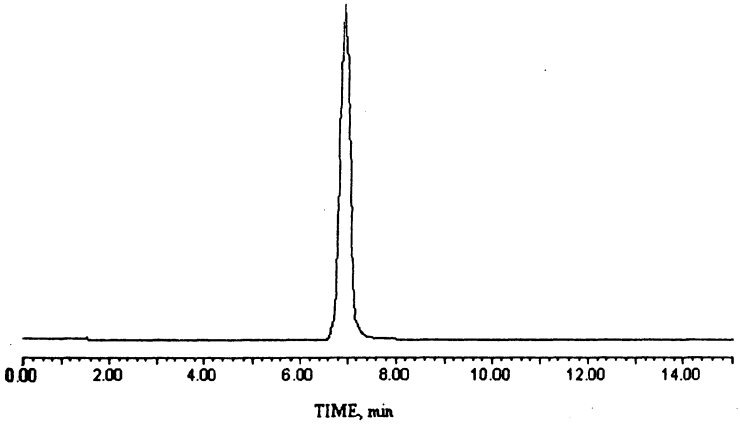


Fig. 4. Chromatogram for determination of CPPU content in samples. Wavelength used for UV detection 254 nm. (Other HPLC conditions as in Operation Conditions)

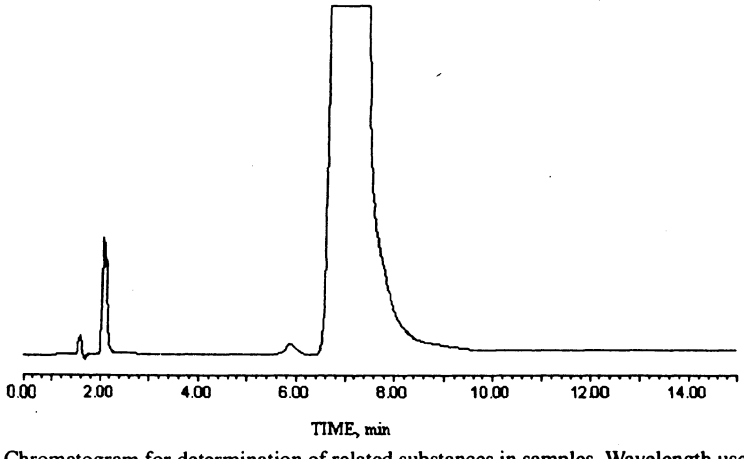


Fig. 5. Chromatogram for determination of related substances in samples. Wavelength used for UV detection 254 nm. Other HPLC conditions as in "Operation Conditions".

TABLE-1
PRECISION OF DETERMINATION (n = 5)

No.	Forchlorfenuron		ACP		DPU	
	Content (%)	RSD (%)	Content (%)	RSD (%)	Content (%)	RSD (%)
1.	99.42	0.20	0.249	0.61	0.01485	0.97
2.	99.57	0.24	0.269	0.72	0.01580	1.50
3.	99.52	0.18	0.258	0.78	0.01567	0.84
4.	99.62	0.18	0.268	0.70	0.01630	0.33
5.	99.61	0.29	0.270	0.54	0.01555	0.97

TABLE-2
RECOVERIES OF FORCHLORFENURON, 4-AMINO-2-CHLORO-PYRIDINE AND N,N′-DIPHENYLUREA (n = 3)

No.	Forchlorfenuron				4-amino-2-chloro-pyridine				N,N′-diphenylurea			
	Initial	Added (mg/mL)	Found	Recovery (RSD), %	Initial	Added (mg/mL)	Found	Recovery (RSD), %	Initial	Added (mg/mL)	Found	Recovery (RSD), %
1.	0.0194	0.010	0.0294	99.70 (0.72)	0.00250	0.00157	0.00413	104.06 (0.88)	0.00015	0.00007	0.00022	97.98 (2.36)
		0.020	0.0395	100.73 (0.11)		0.00314	0.00575	103.37 (0.35)		0.00014	0.00029	101.17 (0.69)
		0.040	0.0594	99.95 (0.29)		0.00628	0.00893	102.26 (0.16)		0.00029	0.00044	100.05 (0.54)
2.	0.0194	0.010	0.0294	99.94 (0.45)	0.00269	0.00157	0.00427	100.60 (0.64)	0.00016	0.00007	0.00021	101.04 (0.59)
		0.020	0.0398	101.75 (0.21)		0.00314	0.00582	101.24 (0.65)		0.00014	0.00030	102.07 (1.50)
		0.040	0.0598	101.05 (1.04)		0.00628	0.00893	99.74 (0.24)		0.00029	0.00045	99.82 (0.20)
3.	0.0197	0.010	0.0299	101.99 (1.04)	0.00259	0.00157	0.00415	99.13 (0.08)	0.00015	0.00007	0.00022	99.63 (0.35)
		0.020	0.0399	100.97 (0.04)		0.00314	0.00580	101.81 (0.35)		0.00014	0.00029	98.82 (0.24)
		0.040	0.0598	100.36 (0.72)		0.00628	0.00896	100.78 (0.56)		0.00029	0.00045	102.61 (1.96)
4.	0.0197	0.010	0.0297	99.83 (0.70)	0.00272	0.00157	0.00427	98.77 (0.21)	0.00017	0.00007	0.00024	100.32 (1.16)
		0.020	0.0396	99.73 (0.04)		0.00314	0.00587	100.31 (0.10)		0.00014	0.00031	103.49 (2.43)
		0.040	0.0597	99.95 (0.60)		0.00628	0.00895	99.17 (0.67)		0.00029	0.00045	99.41 (0.84)
5.	0.0192	0.010	0.0292	99.59 (0.46)	0.00268	0.00157	0.00424	99.59 (0.20)	0.00015	0.00007	0.00023	102.99 (2.37)
		0.020	0.0391	99.66 (0.17)		0.00314	0.00580	99.25 (0.05)		0.00014	0.00030	101.05 (1.73)
		0.040	0.0591	99.82 (0.09)		0.00628	0.00892	99.32 (0.10)		0.00029	0.00044	98.79 (0.65)

Conclusion

It is found that the proposed method in this paper for the determination of forchlorfenuron and its related impurities 4-amino-2-chloropyridine and N,N′-diphenylurea by RP-HPLC-UV detection as well as the identification of these coexisting substances by LC-MS was simple, accurate, reliable, and can be applied to the quality control analyses of the practical industrial and commercial forchlorfenuron samples.

ACKNOWLEDGEMENT

This project was supported by the Analysis & Test Fund of Nanjing University.

REFERENCES

1. T. Okamoto, Y. Isogai, K. Shudo and S. Takahashi, DE 2843722 (1979); US 4193788 (1980).
2. Y. Hou, G.R. Ma, Z.M. Xia and X.B. Zen, *Plant Nutrition and Fertilizer Science*, **25**, 106 (1999).
3. X. Jiang and N. Zhou, *Pesticide*, **35**, 21 (1996).
4. S.R. Lei and L. Luo, *Turang Nonghua Tongbao*, **12**, 55 (1997).
5. D.F. Xu and S.J. Wu, *Jiangsu Pesticide*, **4**, 18 (1999).
6. P.F. Zhu and Y.L. Liu, in: P.Z. Lu, Y.K. Zhang and G.W. Xu (Eds.), Proceedings of 14th National Symposium on Chromatography, Wuxi, China, pp. 287–289 (2003).

(*Received*: 1 September 2004; *Accepted:* 22 March 2005) AJC-4147