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HPLC Determination of Mepiquat Chloride in Commercial Pesticide Formulations

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Abstract A simple, fast and precise High Performance Liquid Chromatographic method with diode array detector was developed and single laboratory validated for the determination of mepiquat chloride in soluble concentrate pesticide formulations. From the results obtained, the repeatability of the method expressed as relative standard deviation (%RSD) was found to be 0.3%, and the limit of detection was 0.02 µg/mL. The accuracy of the whole procedure estimated by the comparison of the results obtained with the reference values, and was found to be acceptable as $t_{\text{cal}} < t_{\text{crit}}$. The precision of the method also considered acceptable as the experimental repeatability relative standard deviation (RSD_r) was lower than the interlaboratory relative standard deviation (RSD_R), calculated by the Horwitz equation.

Keywords Mepiquat chloride · Plant growth regulator · UV–Vis detection · Pesticide formulations

Mepiquat chloride, 1,1-Dimethylpiperidinium chloride, is widely used as plant growth regulator either individually, as mixtures, or together with other pesticides (Tomlin 1997). Mepiquat chloride is a quaternary ammonium pesticide which is very soluble in water, non-volatile, thermally stable, stable in acid and quite stable to hydrolytic or biological degradation (Pico et al. 2000). The European Union has not regulated its level in drinking water and continues applying the values of 0.1µg/L for individual pesticides and 0.5µg/L for total pesticides (EEC 1980). In addition, specific rules in the presence of pesticide residues in processed cereal-based foods and foods for infants and young children are set out in a Commission Directive that states such foods shall not contain residues of individual pesticides at level exceeding 0.01 mg/kg (EEC 1999).

The statement of the concentration of an active ingredient in agrochemical formulations is important for purchasing and using this product. Monitoring the quality of formulations containing mepiquat chloride as active ingredient (a.i.) shows special environmental and analytical interest mainly due to its physicochemical properties. It is well known that the analysis of quaternary ammonium pesticides in water is difficult (Vidal et al. 2004; Worobey 1987). Due to their properties an ion pair can be used in order to be determined by liquid chromatography with diode array detection (Vidal et al. 2004). Many methods have been reported for the determination of mepiquat chloride in environmental samples and food, using different techniques such as high performance liquid chromatography (HPLC), mass spectrometry (MS) (Pico et al. 2000), HPLC-tandem-electrspray-MS (Granby and Vahl 2001), capillary electrophoresis (CE) with UV–Vis or laser-induced fluorescence detection, as well as with new hyphenated techniques like CE-electrospray ionization-MS (Galceran et al. 1997) or ion chromatography with conductivity detection (Fegert et al. 1991), after different clean-up and preconcentration steps. High performance liquid chromatography (HPLC) with UV–Vis detection is one of the most common techniques employed in the analysis and quality control of formulated pesticides, due to the high sensitivity and selectivity achieved (Quintas et al. 2008). Despite of this fact, there are no precedents in the literature about the use of HPLC and UV–Vis detection for the determination of mepiquat chloride in pesticide formulations. Two methods have been

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published for its determination in pesticide formulations until now. The Collaborative International Pesticides Analytical Council (CIPAC) recommends a collaboratively tested method for the analysis of technical, water soluble granules and soluble concentrated products based on the use of ion chromatography with a silica based cation exchange column, an aceton-water-oxalic acid-1,2-diaminoethane mixture as mobile phase and a conductivity detector (CIPAC 2000). This method requires a not common detector in the analysis of pesticide formulations and the use of many chemicals for the preparation of the eluent. There is also an FT-Raman determination (Quintas et al. 2004), which requires instrumentation not easily available in many laboratories.

The objective of this work has been the development of a simple and fast HPLC-UV procedure which could be applied to the routine quality control analysis of formulated products containing mepiquat chloride as active ingredient.

Materials and Methods

Mepiquat chloride analytical standard (99%), Pestanal grade, was provided from Fluka. Mepiquat chloride soluble concentrate (SL) formulations have been obtained from commercial source, accompanied with their certificate of analysis. All the solvents employed were of HPLC grade.

Stock solution of mepiquat chloride was prepared by diluting the appropriate amount of the analytical standard with water (HPLC grade) at concentration 2,500 mg/L. Three working solutions (low calibrated level, medium calibrated level and high calibrated level) were prepared by three independent dilutions of the stock solution with water (HPLC grade) at concentrations of about 0.8, 1.0 and 1.2 times the nominal concentration of a.i. in the formulated product. The freshly prepared working solutions were used for establishing the chromatographic system's precision through repeatability testing and for defining the linearity of response to the analyte.

For the preparation of sample solutions, the appropriate quantity of the commercial SL formulation, containing approximately 1,000 mg (±5%) of a.i., was diluted with water (HPLC grade), so that its concentration fell within the concentration range of the respective working solutions. These samples were used for establishing the precision of the method and for its final evaluation.

The HPLC system used was a Shimadzu UFLC equipped with autosampler (Sil-20AC), degasser, column oven (CTO-20A) and diode array detector (SPD-M20A). The column used was an ODS-AQ (RP, C-18), 4.6 × 150 mm × 5 μm and was purchased from YMC, Inc. The evaluation of HPLC runs and the instrument control was made via LC LabSolutions software. The column temperature was set at 40°C during all runs and the injection volume was 20 μL.

In order to select the most appropriate chromatographic conditions for the HPLC determination of mepiquat chloride, the effect of flow rate, mobile phase composition and detector wavelength were studied. After the optimization, the analysis was carried out with an isocratic method using as mobile phase: 10% :acetonitrile HPLC grade and 90%: 0.03 mM 1-hexanesulfonic acid, sodium salt hydrate (Acros Organics, HPLC grade) in water HPLC grade. The detection wavelength was set at 191 nm. The flow rate was 1 mL/min witch provides pressure of 81 bar.

Results and Discussion

The validation of a new method for the quantitative determination of mepiquat chloride in its SL formulations is presented. CIPAC (CIPAC 1999) guidelines recognize that is necessary to evaluate the following analytical performance parameters: repeatability of injections, linearity of response, precision of the method and the final evaluation of the method made with the comparison of the results obtained during validation with the certified values.

The repeatability of injections was tested, using the medium calibrated level working standard solution, following the selected chromatographic conditions. In case the relative standard deviation (%RSD) of the peak area was $\leq$1%, the repeatability test is considered acceptable. The repeatability of the ten consecutive injections of the experiment was 0.4%, which is in accordance with the precision specification of the auto-sampler.

In order to determine the repeatability of the method, the relative standard deviation (%RSD) was evaluated for nine independent measurements of a sample solution, and found to be 0.3%. The limit of detection (LOD) was 0.02 μg/mL.

The linearity of response to the analyte must be demonstrated at least over the range of nominal analyte concentration ±20% (CIPAC 1999). The linearity of response was determined by analyzing in triplicate three working solutions of the following concentration 549.2 mg/L, 1,098.4 mg/L and 1,647.4 mg/L (Table 1). Linearity is acceptable when the correlation coefficient is $\geq$0.997 (CIPAC 1999). After having performed the multi-point calibration (3 × 3 injections), correlation coefficient (r), standard deviation of relative residuals (S_{rr}), slope and intercept, with confidence intervals at 95% level, were calculated.

Data obtained from the analysis in triplicate of samples from five different batches of the SL mepiquat chloride formulations was used to calculate the experimental $\mathrm{RSD_r}$ values in order to evaluate the precision of the method. The results are considered acceptable if the experimental $\mathrm{RSD_r}$

Table 1 Parameters of linear regression of calibration of mepiquat chloride at 9 calibration levels

Slope (b)	Intercept (a)	r	S_{rr}	Concentration range (mg/L)	$CL_{0.95}$ of a	$CL_{0.95}$ of b
4,132.7	300,260.9	0.9997	0.0077	550–1,650	231,236.4–369,285.3	4,074.5–4,190.9

The linear regression and other calculations were simplified by using ANOVA (Miller and Miller 2000). Confidence interval of 95% was applied for all statistical evaluation

Table 2 : Results of replicate analysis in five different batches

No. of batch	1st m.c.[a] (%w/v)	2nd m.c.[a] (%w/v)	3rd m.c.[a] (%w/v)	Average m.c. (%w/v) ± s[b]	Reference concentration (%w/v)	Statistical evaluation	
1	5.28	5.27	5.28	5.28 ± 0.006	5.21	Bias	0.04
2	5.18	5.19	5 .18	5.18 ± 0.006	5.15	t^{c}_{calc}	2.03
3	5.24	5.24	5.25	5.24 ± 0.006	5.21	SD_{Rlab}	0.0071
4	5.28	5.25	5.25	5.26 ± 0.017	5.21	SD_{Rref}	0.005
5	5.21	5.21	5.22	5.21 ± 0.006	5.19	F_{calc}	2.047
						F_{crit}	6.39
			Average	5.23	5.194		
			Standard deviation	0.037	0.026		

[a] m.c. means measured concentration of analysis in triplicate

[b] Mepiquat concentration found, mean value ± standard deviation of three independent measurements

[c] t_{calc}: experimental t-value calculated from the comparison between HPLC method and the reference value

values are smaller than the values obtained by the modified Horwitz equation (Eq. 2) (CIPAC 1999).

$$RSR_R = 2^{(1-0.5\log C)} \tag{1}$$

$$RSD_r(\%) = RSR_R(\%) \times 0.67 \tag{2}$$

where RSD_r is the repeatability relative standard deviation, RSR_R is the interlaboratory relative standard deviation and C is the concentration of the analyte in the sample expressed as a decimal mass fraction (1 mg/kg = 10^{-6}).The RSD_r value calculated by the Horwitz equation is 2.103, whereas the experimental RSD_r value is 1.05, which is considered acceptable as RSD_r experimental value is lower than the theoretical one.

Five commercially available pesticide formulation products were analyzed using the aforementioned procedure and compared with the reference values. The differences between the measurement concentrations and the reference values given by the manufacturer were not significantly different at probability level of 95% ($t_{crit} = 2.776$) as $t_{calc} < t_{crit}$ (Table 2). The reproducibility of the procedure was good ($F_{calc} < F_{crit}$) (Table 2).

As the results obtained with our procedure and with the validated method of the company did not show any statistically significant differences, and its repeatability and reproducibility was generally good, it was concluded that this method was validated. The decision was made based on the acceptable results of the analysis of additional commercial formulations as part of the long term repeatability and reproducibility tests.

The proposed HPLC method for mepiquat chloride determination in formulated commercial pesticides is fast, because its retention time (RT) is quite short (~1.6 min), in contrast to the CIPAC method (RT = 8–9 min). In addition, the preparation of the eluent of this new method requires fewer components (1-hexanesulfonic acid, sodium salt hydrate) than the CIPAC method (oxalic acid, ethylene diamine). Finally, the detector which was used in this analysis is one of the most common detectors (UV–Vis) employed in HPLC analysis instead of the conductivity detector, suitable for non-suppressed ion chromatography, suggested by CIPAC.

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