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Determination of 3,5 – dimethylpyrazolium glyceroborate nitrification inhibitor in nitrogen fertilizer samples: HPLC-DAD method development and validation for 3,5 – dimethylpyrazole

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ABSTRACT

3,5 – Dimethylpyrazolium glyceroborate is a nitrification inhibitor (a member of pyrazole derivatives) used for the fixation of nitrogen into the soil. In this study, an HPLC-DAD method was developed and validated for determination of 3,5 – dimethylpyrazole in order to determine 3,5 – dimethylpyrazolium glyceroborate in fertilizer samples. For method development, analytical parameters like type of eluent solution and column filling material and device parameters like eluent flow rate, column oven temperature and measurement wavelength were all optimized. For method validation, implementations were performed for linearity, limit of detection (LOD), limit of quantification (LOQ), specificity, stability, intra- and inter-day precision and accuracy. The developed and validated method was used for inhibitor detection in nitrogenous fertilizers. Sample analyses were performed with 95.6–103.3% recovery rates and 0–4.61% relative errors.

1. Introduction

Nitrification is a natural inherent process in soils and includes mostly the transformation mechanisms into ammonium nitrate and then nitrate through nitrosomonas and nitrococus bacteria. Nitrate leaching from agricultural lands and resultant contamination of water resources are major environmental concerns worldwide [1,2]. The nitrogen-use efficiency of agricultural soils has a vital economic and ecological importance, thus nitrification inhibitors are used to mitigate nitrogen loss from the ecosystem [3,4]. Many studies have indicated that nitrification inhibitors could improve the use efficiencies of urea- and ammonium-based N fertilizers and could mitigate soil nitrate leaching and NxO emission through retarding soil nitrification [5–10].

DCD (Diciandiamidine), Nitrapyrin (2-chloro-6-(trichloromethyl)-pyridene) and DMPP (3,4 – Dimethlypyrazolium Phosphate) are the most common and commercial nitrification inhibitors worldwide. 3,5-dimethylpyrazolium glyceroborate has also started to be used for the same purposes. It is produced under the brand name of Doğatech™ in Turkey and classified among nitrogen-inhibiting nitrogenous fertilizers. The inhibitor content varies based on the amount of nitrogen in fertilizer. In these fertilizers, glycerol-boron complex is also used to reduce volatility of 3,5-dimethylpyrazole. This complex is more acidic than weak acids and reduces volatility of alkaline 3,5-dimethylpyrazole [11] through transforming it into a salt (3,5-Dimethylpyrazole glyceroborate) and improves efficiency of new inhibitor formed through bacteria static impact of glyceroboric acid complex.

As far as searched through the literature, there aren't any studies about 3,5-dimethylpyrazole determination. Therefore, the present case was considered as the first case to propose a method for the determination of 3,5-dimetihylpyrazolium glyceroborate recently involved in inhibitor fertilizers. Such a method may also be used for comparison of inhibitors to be developed in future years and for residue studies. Therefore, as an analysis procedure, simple, common and easily-used High Performance Liquid Chromatography (HPLC) method was selected. Initially system variables and analytical parameters of the method were optimized and method validation was performed [12–14]. Validated method was then used to identify inhibitor in nitrogenous fertilizers.

2. Materials and method

2.1. Materials

The 3,5–Dimethylpyrazole, 3,4–Dimethylpyrazole and Dicyandiamide were purchased from Sigma (Taufkirchen, Germany). KH_2PO_4, NaOH, NH_3, HCl, CH_3COOH were purchased from Merck Millipore (Darmstadt,

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Germany). The solutions used in chromatographic tests like ethanol, methanol, acetone, acetonitrile were of chromatographic purity and purchased from Merck Millipore (Darmstadt, Germany). Distilled water used in tests had 18 MΩ resistance and supplied from Millipore Water Purification System (Darmstadt, Germany).

2.2. Apparatus and chromatographic conditions

An Agilent 1260 Infinity HPLC system equipped with a Quat Pump, an autosampler, a degasser, a TCC column heater and a Diode Array Detector (Agilent, Santa Clara, US) were used in analyses. A software system was used for controlling the system and the data processing. The column was Purospher® STAR C-18 analytical column (4.8 mm × 150 mm and 5 µm particle size) (Merck, Darmstadt, Germany). The mobile phase was prepared with acetonitrile and 10 mM K_2HPO_4 aqueous buffer solution (15:85, v/v). A 1.5 ml/min of flow rate and 30°C column temperature was maintained. The measuring wavelength of the detector was adjusted to 210 nm. The injection volume was 20 µl. The column was preconditioned for at least 20 min before the first injection. The running time was set at 20 min.

2.3. Preparation of standards, quality control and sample solutions

To prepare 1000 mg L^{-1} 3,5-Dimethylpyrazole standard solution, 0.100 g solid substance was weighted, dissolved in distilled water, mixed in ultrasonic degasser for 5 min and the resultant volume was completed to 100 ml. To get a calibration curve from this solution, 5, 10, 20, 30, 40 and 50 mg L^{-1} concentration solutions were prepared just ahead of analyses through dilution with distilled water. Quality control (QC) samples were prepared at three concentration levels named as low (5 mg L^{-1}), medium (20 mg L^{-1}) and high (50 mg L^{-1}). These solutions were used to determine intra- and inter-day precision and accuracy. Sample solutions were prepared through dissolving dissolvable fertilizer samples with distilled water. Fertilizer samples were weighted as 10.00 g and dissolved in distilled water. They were mixed in ultrasonic degasser for 5 min and resultant volume was completed to 100 ml. If the concentration of this solution exceeds dynamic range, sample solution was analyzed through dilution with the same method. All solutions were prepared through filtration from 0.45 µm syringe-type (Sartorius, Germany) membrane filter. The calibration standards and QC were prepared freshly and injected into the column at least in triplicates.

2.4. Preparation of fertilizer samples

About 2 kg sack samples were taken from 25 kg fertilizer bags and then about 20 g fertilizer was sampled from each sack. Samples were ground in a porcelain mortar, 10 g ground sample was weighted and dissolved in distilled water. The solution was mixed in ultrasonic bath for 5 min, filtered through blue band filter paper and resultant volume was completed to 100 ml. Before taking into sample vials, solutions were filtered through 0.45 µm teflon syringe-type filter.

For accuracy works, samples were taken from the above specified solutions and analyte was supplemented as to have 5, 10 and 25 mg L^{-1} 3,5-dimethylpyrazole in final volume. Then the solution was again completed to final volume with distilled water. As stock solution, 1000 mg L^{-1} 3,5-dimethylpyrazole solution was used.

2.5. Method validation

The developed method was validated for linearity, limit of detection, limit of quantification (LOD and LOQ respectively), specificity, stability, intra and inter-day precision and accuracy.

A calibration curve was obtained through drawing a graph of changing analyte concentration vs peak areas. To determine the linearity of the method, correlation coefficient (r), slope (b) and intercept (a) values were assessed in y = a + bx linear equation. Then, the calibration graphs with an r value over 0.990 were accepted. For LOD and LOQ values, respectively 3 and 10 times of concentration values corresponding to standard deviations of blank solution creating a signal in the device were calculated and supplemented.

Precision and accuracy works as a part of validation process were performed in two ways as of inter and intra-day. QC samples were prepared at three different concentrations and precision and accuracy values were calculated through the analyses performed within the same day for intra-day and in three different days for inter-day precision and accuracy. Precision value was expressed in Relative Error% (CV%) and accuracy value was expressed in Recovery%.

Specifity is usually carried out to identify whether or not the similar kind of materials possiblye existing in ambient apart from the analyte interfere the analyses. For this purpose, analyte, DMPP and DCD inhibitors were supplemented to fertilizer solutions without any inhibitors and then analyses were formed for specifity.

Stability works were performed in two steps. The first one was "bench-top stability" works. The QC solutions at three different concentrations were analyzed within the same day and their precision and accuracy values were compared. The other step was "stock solution stability" works. The stock solution was kept at −20 °C for 7 days, thawed at room temperature for 6 h and analyzed by using newly prepared standards. The results were compared with the results obtained 7 days ago for precision and accuracy.

3. Result and discussion

3.1. Chromatographic separation and detection

For analyte detection, initially device parameters were tried to be optimized to get the best signals for the analyte through changing column, mobile phase and detector parameters of the device.

For this purpose, the first chromatographic column was optimized. Peaks were not observed with the use of some polar columns. Therefore, apolar columns were used. Among the RP C18 columns used, the best results were obtained from Star RP-18 column. The peaks obtained from this column were smooth in shape, non-tailed and symmetric and analyte holding duration did not change throughout the day. Therefore, the column was found to be appropriate.

Mobile phase composition was prepared in two types as acidic and alkaline. NaH_2PO_4 was used for acidic mobile phase and NH_3 was used for alkaline mobile phase. Although peaks were observed with acidic mobile phase, alkaline phase was not able to yield any peaks. Then besides NaH_2PO_4, organic dissolvers like distilled water, Acetonitrile and Methanol were also used. As the mobile phase composition, the best peaks were obtained from 15% Acetonitrile and 85% 10 mM NaH_2PO_4 aqueous solution. Since flow rate of mobile phase is another parameter, analyte injections were performed at rates between 0,1–1,8 ml min^{-1} and the best results were obtained from 1.5 ml min^{-1} flow rate. Column oven was also used in this method. Although injections were performed at temperatures between 20 and 40 °C, significant effects of temperature were not observed. Chromatographic conditions are provided in Table 1.

3.2. Method validation

For the validation of developed method, linearity, limit of detection, limit of quantification (LOD and LOQ respectively), specificity, stability, intra and inter-day precision and accuracy works were performed. With all these works, method reliability was warranted.

3.2.1. Linearity, limit of detection and limit of quantification

Calibration curve was obtained through drawing the graph of analyte concentrations vs. the signals created in detector by different concentrations of analyte. Method linearity was assessed through

Table 1
Chromatographic conditions.

Parameter	Type or Value
Instrument	Agilent 1300
Detector	Diode Array Detector
Wavelength (nm)	210
Column	Purospher Star RP18e 5 µm (12.5 cm)
Mobile Phase	15% Acetonitrile and 85% aqueous solution of 10 mM of NaH_2PO_4
Flow Rate (mL min^{-1})	1.5
Retention Time (min)	2.28
Injection Volume (µl)	20
Temperature of Column (°C)	30

Table 2
Regression equations and r values.

Days of month	Regression equation	Correlation coefficient
1	y = 36.851x − 0.9524	0.9999
5	y = 35.743x − 3.5714	0.9998
12	y = 36.353x − 1.7234	0.9998

calculating correlation coefficient (r), slope (b), and intercept (a) values of calibration curves obtained at three different days. Regression equations and r values are provided in Table 2. Slope of calibration equations obtained in three different days were quite close to each other. Such a case indicated that method precision did not varied in time. The r values quite close to 1 revealed quite well correlation between the signals obtained from calibration solutions.

The LOD and LOQ values obtained from these calibration equations and 21 blank solutions were respectively calculated as 0.26 and 0.87 mg L^{-1}.

3.2.2. Specifity

Possible interference of salt and organic compounds and other commercially available inhibitors like DCD and DMPP were investigated. For this purpose, initially to investigate the interference level of fertilizer matrix, 10 g of analytical grade Urea, $(NH_4)_2SO_4$, NH_4NO_3, KNO_3, K_2SO_4, $NH_4H_2PO_4$, $Ca(NO_3)_2{\cdot}4H_2O$ and $Mg(NO_3)_2{\cdot}6H_2O$ were weighted, dissolved in distilled water, 3,5-dimethylpyrazole was added as to have 20 mg L^{-1} concentration in final volume and resultant solution was mixed in an ultrasonic bath for 5 min. Final volume was then completed to 100 ml. The signals obtained from these solutions were compared with the signals obtained from QC solutions. Results are provided in Table 3. A recovery ratio of between 98.0–104.5% was attained in a matrix environment of 100 g L^{-1}concentration of chemical substances potentially to be produced as inhibitor fertilizer.

Commercially available DCD and DMPP inhibitors were supplied from the market at analytical quality. 25 mg L^{-1} solutions of DCD, DMPP and 3,5-dimethylpyrazole were prepared and they were injected both separately and in mixtures to get peaks from these solutions. When DCD was used, peaks were not observed throughout 20 min reading period. With DMPP on the other hand, a peak with an average retention time of 2.41 min and just following 3,5-dimethylpyrazole but not coinciding with it was observed. Results obtained from mix solution of three inhibitors are presented in Fig. 1. All such outcomes revealed that the present method was free of any interferences effects of commercially available inhibitors and salts.

3.2.3. Precision and accuracy

Precision and accuracy works of the method were implemented at low, medium and high concentrations (respectively as 5, 25 and 50 mg L^{-1}). In each test, average of 5 injections was presented as the results. Intra and inter-days precision values were assessed through taking peak areas and retention times into consideration. Results are provided in Table 4. According to the table, at three different concentration levels, intra-days precision (CV%) values varied between 0.35–0.76 for t_R and between 0.85–1.24 for mAU. Inter-days precision (CV%) values varied between 1.78–2.60 for t_R and between 1.2–2.57 for mAU. For accuracy (%), recovery% values varied between 99.12 and 102.44.

3.2.4. Stability

The solutions prepared for bench-top stability were analyzed by using newly prepared standards in the morning and evening of the same day. Resultant precision and accuracy values were compared. The solution prepared for stock solution stability were preserved at the same concentration levels at −20 °C for 7 days, then thawed at room temperature for 6 h and analyzed by using again the newly prepared standards. Resultant outcomes were compared for precision and accuracy with the results obtained 7 days ago. The results presented in Table 5 revealed a decrease in precision and accuracy values with the time passed from the preparation of the solutions. However, these values for RE% and CV% were maximum 4.0 and 4.2, respectively.

3.3. Application of fertilizer samples

The developed and validated method was applied to detect 3,5-dimethylpyrazolium glyceroborate in fertilizer samples. The inhibitory content of these samples was calculated using the stoichiometric ratio of 3,5-dimethylpyrazole. The formula of the calculation is given in Eq. (1);

$$W_{DMPB}\% = \frac{C_{DMP} \times V_{sol} \times df \times 1.635}{m_f \times 10} \quad (1)$$

where; C_{dmp} is the concentration of 3,5-dimethylpyrazole in sample solution (mg L^{-1}), df is a dilution factor, V_{sol} is the volume of fertilizer solution (liter), m_f is weight of fertilizer sample (gram), 1.635 is the stoichiometric factor from DMP to DMPB.

For this purpose, the fertilizers supplied from Doğatech ArGe Co., producing nitrification-inhibiting fertilizers, were prepared through

Table 3
Effects of interfering substances on the determination of 3,5-Dimethylpyrazole (n = 5).

Interferent	Retention Time (min)	Added (mg L^{-1})	Found (mg L^{-1})	R%	CV%
Urea	2.28	20.0	20.1 ± 0.2[a]	100.5 ± 1.0	1.0
$(NH_4)_2SO_4$	2.27		20.9 ± 0.2	104.5 ± 1.0	1.0
NH_4NO_3	2.28		19.6 ± 0.1	98.0 ± 0.5	0.5
KNO_3	2.28		20.0 ± 0.2	100.0 ± 1.0	1.0
K_2SO_4	2.27		19.9 ± 0.1	99.5 ± 0.5	0.5
$NH_4H_2PO_4$	2.26		19.8 ± 0.1	99.0 ± 0.6	0.6
$Ca(NO_3)_2{\cdot}4H_2O$	2.28		20.2 ± 0.3	101.0 ± 1.5	1.5
$Mg(NO_3)_2{\cdot}6H_2O$	2.29		20.1 ± 0.1	100.5 ± 1.0	1.0

[a] x ± s.

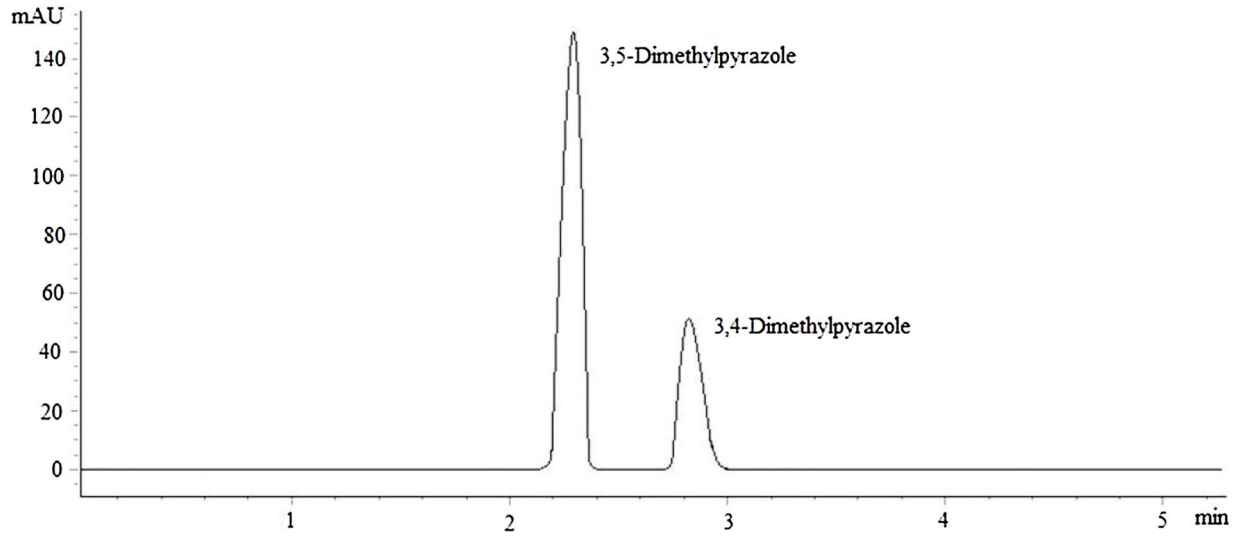

Fig. 1. Chromotgram showing the retention peaks for 10 mg l^{-1} DMPP (3,4-Dimethylpyrazole) and 10 mg l^{-1} 3,5-Dimethylpyrazole obtained with the newly developed method: the first peak is analyte peak and the second one is interferent peak (DCD inhibitor did not retain in the column).

Table 4
Precisions values of retention time (tR) and peak area (mAU) and coefficient of variation (CV%) Intra- and inter-day, accuracy values as a percentage value of the measured concentration (n = 5).

QC levels	Intra-days precision (CV %).		Inter-days precision (CV %).		Accuracy (%).
	t_R	mAU	tR	mAU	
Low	0.76	1.24	2.37	2.46	102.44
Medium	0.35	0.85	2.60	1.23	100.20
High	0.42	0.97	1.78	2.57	99.12

dissolving in distilled water since the analyte has high water solubility. Since possible interference of fertilizer matrixes were previously tested, all fertilizer solutions were prepared at 100 g L^{-1} concentration. Since the solutions prepared from Stable N-21, Stable N-46, Stable N-36, Basic 18-18-5, Basic 25-10-0 and Basic 14-14-17 fertilizers have high nitrogen contents and thus high inhibitor contents, they were diluted 20 times before the analyses and the other fertilizer samples were diluted 2 times. Analyte additions were also performed based on these dilution rates. Matrix and analyte concentrations, sample analysis, addition, recovery and relative error results are provided in Table 6. The results of sample analysis and Recovery% are given as DMPB. The recovery (%) was calculated as the ratio of resultant concentration to the total of added and producer declared concentrations. Relative Error (%) was calculated as the ratio of the difference between theoretical and experimental concentrations to theoretical concentration (Theoretical concentration:total of non-added and added concentrations, experimental concentration: the concentration after addition). The Recovery % values varied between 96.0 − 107.9% and Relative Error% values varied between 0 and 5.16%.

4. Conclusions

The present method was developed to determine DMPB in nitrification-inhibiting fertilizers. For this purpose, DMPB contents of the fertilizer samples were calculated using the determination of 3,5-dimethylpyrazole with HPLC. This is the first study performed for the detection of relevant analyte. Therefore, the method developed for 3,5-dimethylpyrazole detection with HPLC was initially validated and then used for analyses on fertilizer samples. In analyses, matrix ambient of available inhibiting fertilizers was tested for possible interferences of fertilizer matrixes and interferences were not observed. Interference effects of active ingredients of the other inhibiting fertilizers like DMPP and DCD were also investigated. While DCD was not observed in chromatogram, DMPP was observed at a different point from the analyte peak in a discernable fashion.

The developed method is a rapid and easy-to-apply method with high precision, accuracy and selectivity. With these superior attributes, the method was successfully applied to fertilizer samples. Resultant outcomes match up with producer-declared values and addition-recovery values were quite satisfactory.

Table 5
Results for stability analyses performed as bench-top stability andstock solution stability (n = 3).

Stability	Analyte Concentration (mg L^{-1})	Morning	Evening	7 days later	Accuracy (RE%)	Precision (CV%)	
						Before	After
Bench-top	5	5.0 ± 0.1[a]	4.9 ± 0.1	–	2.00	2.0	2.1
	20	19.8 ± 0.2	20.1 ± 0.3	–	1.52	1.0	1.5
	50	49.6 ± 0.2	49.0 ± 0.4	–	1.21	0.4	0.8
Stock Solution	5	5.0 ± 0.1		4.8 ± 0.2	4.00	2.0	4.2
	20	19.8 ± 0.2		19.3 ± 0.4	2.53	1.0	2.1
	50	49.6 ± 0.2		48.7 ± 0.5	1.81	0.4	1.0

[a] x ± s.

Table 6
Analysis results of fertilizer samples (n = 3).

Product	Matrix	Producer declared DMPB content		Added DMPB (mg L^{-1})	Found DMPB(mg L^{-1})	R%	RE%
		In fertilizer (%)	In solution (mg L^{-1})				
Stable N-21	$(NH_4)_2SO_4$	0.22	11.0	–	11.1 ± 0.2[a]	–	–
				5.0	16.0 ± 0.3	99.4 ± 1.9	0.63
				10.0	21.2 ± 0.5	100.5 ± 2.4	0.47
				25.0	35.8 ± 0.8	99.4 ± 2.2	0.60
Stable N-46	Urea	0.57	28.5	–	28.2 ± 0.3	–	–
				5.0	33.7 ± 0.7	101.5 ± 2.1	1.48
				10.0	38.8 ± 0.7	101.6 ± 1.8	1.57
				25.0	53.1 ± 1.1	99.8 ± 2.0	0.19
Stable N-36	$(NH_4)_2SO_4$Urea	0.39	19.5	–	19.2 ± 0.3	–	–
				5.0	24.3 ± 0.7	100.4 ± 2.9	0.41
				10.0	29.1 ± 0.8	99.7 ± 2.7	0.34
				25.0	44.4 ± 1.5	100.5 ± 3.4	0.55
Stable K-38	K_2SO_4, $(NH_4)_2SO_4$	0.05	25.0	–	24.4 ± 0.5	–	–
				5.0	31.0 ± 0.6	103.3 ± 1.9	3.28
				10.0	34.7 ± 0.5	99.1 ± 1.4	0.86
				25.0	49.4 ± 1.0	98.8 ± 2.0	1.21
Stable P-46	$NH_4H_2PO_4(NH_4)_2SO_4$	0.04	20.0	–	20.5 ± 0.5	–	–
				5.0	24.8 ± 0.5	97.3 ± 2.0	2.82
				10.0	30.6 ± 0.6	100.3 ± 1.9	0.30
				25.0	44.7 ± 0.9	98.2 ± 2.0	1.79
Basic 18-18-5	$NH_4H_2PO_4K_2SO_4$, $(NH_4)_2SO_4$	0.19	8.5	–	9.0 ± 0.3	–	–
				5.0	13.9 ± 0.4	99.3 ± 2.9	0.72
				10.0	19.0 ± 0.4	100.0 ± 2.1	0
				25.0	32.5 ± 0.7	95.6 ± 2.2	4.61
Basic 25-10-0	$NH_4H_2PO_4(NH_4)_2SO_4$ Urea $Ca(NO_3)_2$, Mg $(NO_3)_2$	0.26	13.0	–	14.0 ± 0.1	–	–
				5.0	18.4 ± 0.2	96.8 ± 1.1	3.23
				10.0	24.0 ± 0.8	100.0 ± 3.3	0
				25.0	39.1 ± 0.6	100.3 ± 1.5	0.26
Basic 14-14-17	$NH_4H_2PO_4$ K_2SO_4, $(NH_4)_2SO_4MgSO_4$	0.15	7.5	–	7.2 ± 0.3	–	–
				5.0	12.5 ± 0.4	102.5 ± 3.3	2.45
				10.0	17.3 ± 0.6	100.6 ± 3.5	0.58
				25.0	32.0 ± 0.6	99.4 ± 1.8	0.63

[a] x ± s.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] H.J. Di, K.C. Cameron, Reducing environmental impacts of agriculture by using a fine particle suspension nitrification inhibitor to decrease nitrate leaching from grazed pastures, Agric. Ecosyst. Environ. 109 (2005) 202–212.
[2] G.D. Liu, W.L. Wu, J. Zhang, Regional differentiation of non-point source pollution of agriculture-derived nitrate nitrogen in groundwater in northern China, Agric. Ecosyst. Environ. 107 (2005) 211–220.
[3] D.L. Dinnes, D.L. Karlen, D.B. Jaynes, T.C. Kaspar, J.L. Hatfield, T.S. Colvin, C.A. Cambardella, Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils, Agron. J. 94 (2002) 153–171.
[4] G. Subbarao, O. Ito, K. Sahrawat, W. Berry, K. Nakahara, T. Ishikawa, T. Watanabe, Scope and strategies for regulation of nitrification in agricultural systems –challenges and opportunities, Crit. Rev. Plant Sci. 25 (2006) 303–335.
[5] W. Linzmeier, R. Gutser, U. Schmidhalter, Nitrous oxide emission from soil and from a nitrogen-15-labelled fertilizer with the new nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), Biol. Fertil. Soils. 34 (2001) 103–108.
[6] H.J. Di, K.C. Cameron, The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland, Soil Use Manage. 18 (2002) 395–403.
[7] D. Hatch, H. Trindade, L. Cardenas, J. Carneiro, J. Hawkins, D. Scholefield, D. Chadwick, Laboratory study of the effects of two nitrification inhibitors on greenhouse gas emissions from a slurry-treated arable soil: impact of diurnal temperature cycle, Biol. Fertil. Soils 41 (2005) 225–232.
[8] M. Zaman, S. Saggar, J.D. Blennerhassett, J. Singh, Effect of urea and nitrification inhibitors on N transformation gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system, Soil Biol. Biochem. 41 (2009) 1270–1280.
[9] D. Chen, H.C. Süter, A. Islam, R. Edis, Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea, Soil Biol. Biochem. 42 (2010) 660–664.
[10] X.X. Dong, L.L. Zhang, Z.J. Wu, D.P. Li, Z.C. Shang, P. Gong, Effects of the nitrification inhibitor DMPP on soil bacterial community in a Cambisol in northeast China, Soil Sci. Plant Nutr. 13 (2013) 580–591.
[11] N. Sundaraganesan, E. Kavitha, S. Sebastian, J.P. Cornard, M. Martel, Experimental FTIR, FT-IR (gasphase) FT-Raman and NMR spectra, hyper polarizability studies and DFT calculations of 3,5-dimethylpyrazole, Spectrochim. Acta Part A 74 (2009) 788–797.
[12] R. Rathore, J.P. Jain, A. Srivastava, S.M. Jachak, N. Kumar, Simultaneous determination of hydrazinocurcumin and phenol red in samples from ratintestinal permeability studies: HPLC method development and validation, J. Pharm. Biomed. Anal. 46 (2008) 374–380.
[13] B. Blanchet, B. Billemont, J. Cramard, A.S. Benichou, S. Chhund, L. Harcouet, S. Ropert, A. Dauphin, F. Goldwasser, M. Tod, Validation of an HPLC-UV method for sorafenib determination in human plasma and application to cancer patients in routine clinical practice, J. Pharm. Biomed. Anal. 49 (2009) 1109–1114.
[14] P. Giuliani, M. Zuccarini, S. Buccella, M. Rossini, I. Alimonte, R. Ciccarelli, M. Marzo, A. Marzo, P. DiIorio, F. Caciagli, Development of a new HPLC method using fluorescence detection without derivatization for determining purine nucleoside phosphoryl aseactivity in human plasma, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1009–1010 (2016) 114–121.