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RESEARCH & DEVELOPMENT OF P_2O_5 DETERMINATION METHOD IN PHOSPHATE FERTILIZERS AND ITS VALIDATION

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ABSTRACT

The validation of chemical measurement for Phosphorus determination in fertilizers followed a chain of experimental procedures to ascertain the performance characteristic (PC), connecting Precision, accuracy, reproducibility, recovery, linearity, limit of detection and of quantification. Performance characteristics are accomplished to ensure that analytical method fits the purpose of particular testing. This method was conducted in three stages those were preparation, testing and data processing. The standard test method for determination of P2O5 content in fertilizers used for regulatory purpose is somewhat lengthy and time taking. A modification was done to the standard method to create a more efficient approach that meets validation criteria and is suitable for analyzing advisory fertilizer samples. During the year 2023-24, comprehensive research and development activity were carried out at Soil and Water Testing Laboratory (SWTL) for Research, Faisalabad and analytical data was generated. The obtained results were processed statistically and compared with the acceptance requirements. The coefficient of determination (R^2), precision and accuracy/recovery percentage respectively were 0.9996, 0.3-1.10% and 98.01-102.34%, while LOD and LOQ were 0.84 and 2.25%. Based on the results the study concluded that the modified test method applied to determine $P_2O_5$was appropriate for its intended use based on the acceptance criteria of performance characteristics set forth in the validation protocol and can be applied as routine method at laboratory.

Keywords: Phosphate, Fertilizer, validation, LOD, LOQ, Nitrophos, DAP

INTRODUCTION

There are two kinds of analytical methods to determine phosphorus: standard and non-standard. The first method is developed, validated, peer-reviewed and published by respective regulatory bodies, such as national, regional, or international organizations. The ISO 17025:2017, clause 3.8, asserts that validation for an analytical method should fulfill specified requirements that are adequate for the intended use. The terms validation and quality assurance (QA) are widely used. Validation of an analytical method is essential in controlling the quality of quantitative analysis. Validation can be defined as the process by which laboratory studies establish that the analytical parameters of the method meet the requirements for the intended analytical applications (Shehata et al., 2018). Method validation is accomplished through the assessment of various analytical attributes, including repeatability, reproducibility, method working range, precision, detection limit (LOD), quantification limit (LOQ) and recovery (Sahoo et al., 2018). The ISO definition of validation is confirmation by examining and providing objective evidence that the particular requirements of a specified intended use are fulfilled. Soil and Water Testing Laboratory (SWTL) for Research at AARI, Faisalabad, is working as a divisional lab under the Umbrella of Soil Fertility Research Institute Lahore, where different kinds of fertilizers are being tested for their quality. For the determination of P2O5in Phosphate fertilizers such as Single Super Phosphate (SSP), Diammonium Phosphate (DAP), Nitrophos (NP), and other combined fertilizers (NPK), the test method used was published in Pakistan Standard PS No. 67-1996. However, in this study, only one step of precipitation time for the settlement of yellow precipitates was modified, and test results were characterized to ensure that they fulfil the

typical validation criteria. The validated method of analysis results is vital for achieving superior quality (Arkaban et al., 2021; Striegel, 2021). The aim of the study was to validate a modified titration method for the analysis/determination of Phosphate fertilizer samples with ease, saving time and facilitating more clients/consumers in less time.

MATERIALS & METHODS

During this methodology development, activity on 6.12.2023 analysis was performed as per the standard procedure of P2O5determination given in PS No. 67-1996. In this method, Yellow precipitate of ammonium phosphomolybdate forms depending on the concentration of phosphorus present in the particular fertilizer sample that is stayed for one night. The rest of the procedure is completed the next day. In our modified method/ parallel method, precipitation settling time was kept for three hours in the refrigerator, and the rest of the procedure was completed on the same day. Samples were analyzed by two different analysts (Analyt-1 and Analyst-2).**Products Tested:** Analysis was performed while selecting five different kinds of fertilizers given below;

SSP (P_2O_5 18%)
DAP (P_2O_5 46%)
Nitrophos (P_2O_5 20%)
NPK Solid (P_2O_5 18%)

Performance Characteristics

Method Precision: The precision of a method is the closeness of independent results attained under required conditions. It was computed as the results' relative standard deviation (%RSD) (AOAC, 2016).

Precision: RSD= CV= (ð/x)×100

Method Accuracy: It is the closeness of concurrence between a test result and the established reference value. The recovery percentage can be used as a criterion to express the method's accuracy (AOAC, 2016). Four different samples were analyzed for available P2O5. The total recovery percentage was calculated.

Accuracy / Recovery = (X/K)×100

Reproducibility: Reproducibility is the nearness of agreement between independent outcomes achieved with the same method on matching test material obtained under the same circumstances. In this method validation process, repeatable conditions were created by analyzing the samples by the same analyst at short intervals of time.

Linearity: Linearity is found out by determining a sequence of standards of stock solution/diluted stock solution using at least seven various concentrations in range of 50-150% of the anticipated working range (Rao, 2018).

Limit of Detection (LOD): It is the minimum quantity of any analyte in the sample, which is detectable but not essentially quantified (Bakircioglu et al, 2011; González et al., 2018).

LOD = 3.3× SD of intercept/slope

Limit of Quantification (LOQ): It is the minimum quantity of any analyte in the sample, which is detectable, but not essentially quantified (González et al., 2018). It is calculated based on Standard Deviation of response and slope of Calibration Curve.

LOQ = 10 × SD of intercept/slope

Measurement of uncertainty: The estimation process includes five stages that are specific determination, identify source of uncertainty, define raw uncertainties, the merger of raw uncertainties, and calculation uncompleted uncertainties (Wiyantoko et al. 2018). This may be attributed to several factors including method, personal, environment, chemicals and equipment used. The blend of all the affecting factors is said to be combined uncertainty. The budget of uncertainty is comprises of whole the uncertainties because of above cited factors (EURACHEM/CITAC 2015). Under ISO/IEC: 17025 the testing laboratories must correspond to their uncertainties with definite confidence level which is named as expanded uncertainty (Aslam et al., 2021).

Combined uncertainty =

$$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + \ldots + u(x_n)^2}$$

Expanded uncertainty = Combined uncertainty ×K

The desired confidence level is the deciding factor for picking the factor k.

For an estimated 95% confidence level, k is 2.

RESULTS

Various performance characteristics, including accuracy, precession, reproducibility, repeatability, detection and quantification limit, linearity, and measurement uncertainty, were approximated to validate the method for determining P2O5 in fertilizer samples.

Method Precision: Precision was computed as the relative standard deviation (%RSD) of the results and given in Tables 1and2, respectively, by analysts 1 and 2. This was carried out via repeatability along with data reproducibility. According to "AOAC International 2016, the "Expected Precision %RSD (RSDr %)" as function on analyst must be less than 1.9 of the analyst. All test samples tested with STM were found with RSDr% less than 1.0 (Table No. 1& 2). Hence, the method proved itself very precise.

Method Accuracy: This was computed through the calculation of recovery percentage. Taverniers et al. (2010) reported the acceptable range of the recovery is 95% to 105% for analyte concentration of 1µg/mL. In the present method validation the recorded recovery of P_2O_5 content in all kind of fertilizer samples analyzed by two analysts was retained within suggested range of criteria (Table 1& 2), hence, the method is verified in this performance characteristic and is marked as qualified.

Table. 1: Repeated measurements of samples by Analyst I

R/N	DAP	Nitrophos	SSP	Solid NPK
1	45.7	19.68	17.67	18.48
2	45.35	19.38	17.72	18.51
3	45.46	19.66	17.68	18.5
4	45.4	19.46	17.89	18.4
5	45.36	19.45	17.36	18.45
6	45.7	19.64	17.66	18.65
7	45.36	19.84	17.84	18.32
8	45.4	19.65	17.54	18.44
9	45.6	19.68	17.68	18.11
10	45.52	19.57	17.65	18.36
Average (X)	45.49	19.60	17.67	18.42
SD (Ur)	0.138	0.137	0.147	0.142
Accuracy/recovery	98.88	98.01	98.16	102.34
Precision (%RSD)	0.30	0.70	0.83	0.77

Table2. Repeated measurements of samples by Analyst 2

R/N	**DAP**	**Nitrophos**	**SSP**	**Solid NPK**
1	45.2	19.24	17.45	18.15
2	45.22	19.48	17.72	18.4
3	45.4	19.38	17.68	18.5
4	45.4	19.36	17.89	18.4
5	45.3	19.45	17.36	18.45
6	45.7	19.48	17.85	18.36
7	45.61	19.84	17.69	18.27
8	45.4	19.52	17.89	18.22
9	45.26	19.3	17.96	18.21
10	45.6	19.57	17.65	18.14
Average (X)	45.41	19.46	17.71	18.31
SD (Ur)	0.175	0.167	0.195	0.128
Accuracy/recovery	98.72	98.31	98.41	101.72
Precision(%RSD)	0.39	0.86	1.10	0.70

Reproducibility: Reproducibility data relating to two analysts carrying out P_2O_5 determination analysis by titration method at various times was acquired by the application of F-test which exhibited that the F calculated for all kinds of fertilizers is less than F-critical (3.178). Consequently, the results were insignificant and the method was acceptable

Table. 3. F-Test of Two Variables (Analyst 1 vs Analyst 2)

	Analyst 1	*Analyst 2*	*Analyst 1*	*Analyst 2*	*Analyst 1*	*Analyst 2*	*Analyst 1*	*Analyst 2*
	DAP		*SSP*		*Nitrophos*		*Solid NPK*	
Mean	45.49	45.41	17.67	17.60	19.71	19.46	18.42	18.31
Variance	0.030588	0.01905	0.037982	0.021499	0.027929	0.018832	0.020262	0.016511
Observations	10	10	10	10	10	10	10	10
Df	9	9	9	9	9	9	9	9
F	1.605658		1.766706		1.483037		1.227187	
P(F<=f) one-tail	0.245794		0.204688		0.283259		0.382684	
F Critical one-tail	3.178893		3.178893		3.178893		3.178893	
Pass/Fail	Pass		Pass		Pass		Pass	

Linearity: The linearity graph was plot by means of Microsoft Excel and computer software (Concentration vs. Peak Area Response). The correlation coefficient r^2 was calculated as well. It was found that the correlation coefficient r^2 was 0.9996

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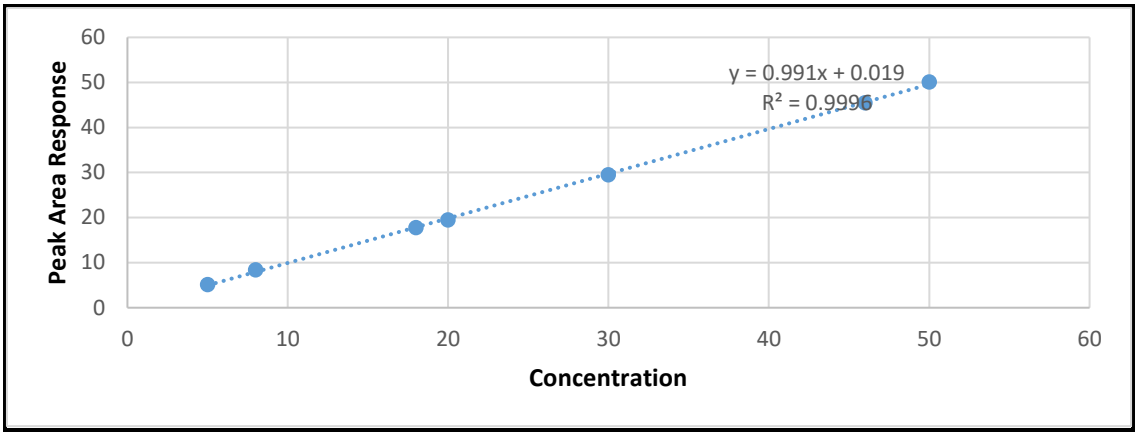

Figure 1. Concentration vs. Peak Area Response

Limit of Detection & Quantification: In this study of method validation, the Limit of Detection (LOD) was found to be 0.84, and the limit of quantification was determined to be 2.55. Hence, we can say that this method is suitable for determining 0.84% to 50% of the concentration of analyte in different kinds of phosphate fertilizer samples.

Measurement of uncertainty: The uncertainty budget comprised of 2 kinds of uncertainty sources (A & B). The uncertainty of the studied method was ±0.0187316425 at 95% confidence interval (Table 4).

Table 4. Calculation of Uncertainty

S/N	Sources of Uncertainty	Uncertainty	Type A/B	K Factor (Where Applicable)	Uncertainty Contribution	Average or Value	Relative Uncertainty	Combining Uncertainty
1	Repeatability	0.0251	A	1	0.0251	45.16	0.0006	0.000000
2	Reproducibility	0.0108	A	1	0.0108	45.16	0.0002	0.000000
3	V. Flask (100ml)	1	B	2	0.5000	100	0.0050	0.000025
4	V. Flask (500ml)	2	B	2	1.0000	500	0.0020	0.000004
5	V. Flask (1000ml)	5	B	2	2.5000	1000	0.0025	0.000006
6	Cylinder 50 ml	0.5	B	2	0.2500	50	0.0050	0.000025
7	Pipett (01 ml)	0.01	B	2	0.0050	1	0.0050	0.000025
8	Pipett (05 ml)	0.01	B	2	0.0050	5	0.0010	0.000001
9	Pippet (10 ml)	0.02	B	2	0.0100	10	0.0010	0.000001
10	Digital burette (50 ml)	0.02	B	2	0.0100	50	0.0002	0.000000
11	Analytical balance	0.1	B	2	0.0500	200	0.0003	0.000000
Combined Uncertainty (Uc)		0.00936582	@	68 % CL				
CL (K)		2	=	95% CL				
Expanded Uncertainty (Ue)		0.01873164	@	95% CL				

DISCUSSION

Full validation was made for the modified analytical method used to analyze P2O5 content in four different kinds of Phosphate fertilizers (DAP, Nitrophos, SSP, and solid NPK) samples by two analysts at SWTL Faisalabad laboratory. It was proved that the adopted modified method was reliable for such measurement. Also, the validated method presented in this paper meets the requirements and the criteria set in EURACHEM (2000)- A Laboratory Guide to Method Validation and Related Topics and other international regulations for the method validation. In comparison with a previously established method of P2O5determination as per PS No. 67-1996, where the yellow precipitate of ammonium phosphomolybdate in the specific fertilizer sample stayed for one night, in our modified method/ parallel method, precipitation settling time was kept 3 hours in the refrigerator and rest of the procedure remained same. The coefficient of determination (R2), precision and accuracy/recovery percentage respectively were 0.9996, 0.3-1.10% and 98.01-102.34%, while LOD and LOQ were 0.84 and 2.25%. Based on the study results, we can say that our method is suitable for determining P2O5 in different kinds of phosphate fertilizer samples.

CONCLUSION

This validation study concluded that the test method with the modified step was appropriate for its intended use based on the performance characteristics in this manuscript and the acceptance criteria in the table below.

Table. 5 Summary of Performance Characters

Sr. No	Performance Characters	Range of value	criteria	Conclusion
1	Precision (%RSDr)	0.3-1.1	<1.3%	Pass
2	Accuracy (Recovery)	98.01-102.34	98-105	Pass
3	Reproducibility /F-Test		$F_{Cri.} > F_{Cal.}$	Pass
4	LOD	0.84	< 5.0 Excellent <10 Acceptable	Pass
5	LOQ	2.55	< 10 Excellent <15 Acceptable	Pass

AUTHORS' CONTRIBUTION

Authors at sr. No. 1& 2 involved in analysis of fertilizers and statistical parameters of the manuscript.

Sr. no. 3, 4 & 5 in write up of discussion and references.

AUTHORS CONFLICT STATEMENT

All the authors have no conflict of interest.

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