

AN IMPROVED METHOD FOR THE DETERMINATION OF SOIL 'AVAILABLE' PHOSPHORUS*

by J. R. BURROWS

South African Sugar Association Experiment Station, Mount Edgecombe

Abstract

A routine discrete sample method for the determination of soil 'available' phosphorus, using manual or automated colour complex preparation, is described. After extraction, orthophosphate is determined colorimetrically using a stable 12-molybdoantimonylphosphate complex. This improved method gives results comparable to those obtained using the existing Dickman and Bray procedure. When used with a discrete sample automatic analyser system, a labour saving of about seven man-hours per 200 samples is effected. The development of this method and its adaptation for use with the LKB sample processor, together with the steps necessary to minimise adverse effects of sample carryover, are discussed.

Introduction

Phosphorus (as orthophosphate) is an essential plant nutrient and assessing the quantity of this element in the soil which is available to sugarcane, forms an important part of the work of the Fertilizer Advisory Service (FAS) of the S.A. Sugar Association Experiment Station. Demand for the analytical services of the FAS laboratory has progressively increased over the past few years. During the 1976-77 season over 17 000 'available' phosphorus determinations were made, constituting about 11 percent of the total laboratory workload.

Sugarcane response to fertilizer phosphate has been found to correlate well with the orthophosphate content of dilute acid soil extracts (du Toit⁷). After extraction and separation, 'available' phosphorus was determined colorimetrically with a Klett-Summerson colorimeter using a molybdophosphate complex reduced with tin (II) chloride (Dickman and Bray⁶). This time-consuming procedure was improved with the introduction of an LKB 7400 calculating absorptiometer to speed up the measurement of colour complexes (Burrows and Meyer⁴) but the laborious manual preparation of colour complexes still remained as a major factor limiting higher sample throughput.

An LKB 2071 sample processor has been successfully used to prepare samples for other determinations (Burrows³, Burrows and Meyer⁴) but it was not possible to adapt the manual Dickman and Bray procedure for use with this equipment. The high extractant to sample ratio (50:1) results in a low orthophosphate concentration in the extract. This factor, combined with the relative insensitivity of the colour complex, meant that a large volume of sample in relation to that of reagent was needed to achieve some measure of sensitivity. The LKB sample processor uses interlinked sampling and diluent pumps. Metered quantities of the first reagent/diluent are used as a rinse to purge portions of sample adhering to the inside of the sampling probe and connecting pipe. The effectiveness of this operation depends on the relative quantity of sample and diluent used. Information presented with the sample processor (LKB application notes⁹) indicates that carryover from incomplete sample rinsing increases rapidly when the diluent to sample ratio is reduced below 4:1. The Dickman and Bray⁶ procedure with a total diluent to sample volume ratio of 0,44:1 was therefore clearly unsuitable for automatic preparation with this equipment.

A more sensitive colour complex was therefore required for the successful development of an automatic preparation procedure. Additional important features were:

- the complex should have an absorbance peak in the range 400-700nm, (the effective working range of the LKB 7400 calculating absorptiometer)
- it should be stable for at least 30 minutes after formation
- if possible, all reagents should be capable of being mixed and added as a single solution.

Murphy and Riley¹⁰ recommended a procedure involving the use of a 12-molybdoantimonylphosphate complex which was thought to be suitable. The complex has a main absorbance peak at 882nm with a side peak at 700nm. No details concerning the use of this side peak were given, but calculations from the absorbance spectra presented by Murphy and Riley¹⁰ showed that this peak was about 40% more sensitive than that of the existing colorimetric procedure.

In this paper is described the development of Murphy and Riley's¹⁰ method into an automated procedure suitable for determining 'available' phosphorus in soils using the LKB 2071 sample processor and LKB 7400 calculating absorptiometer.

Material and Methods

Reagent grade chemicals and deionised water are used throughout.

Sample preparation

Weigh 5 g of soil and transfer it to a bottle suitable for the end-over-end shaker used. Add 250 ml of 0,01M sulphuric acid (prepared by diluting 98% sulphuric acid). Stopper the bottle with a well-fitting cork and shake for half an hour at 22 revolutions per minute.

- Filter the extract immediately through a Whatman No. 1 filter paper into a clean glass container. Include one blank extract (without soil) with every batch.

OR

- Transfer 10 ml of the extract to a pyrex test tube using an Oxford 5-10 ml transfer pipette. Centrifuge for 10 mins in the MSE G8 centrifuge using the multitube carriers.

OR

- Transfer 3 ml of the extract to an LKB preparation tube held in a sample rack using an Oxford 15 ml transfer pipette. Centrifuge for 10 mins in the MSE G8 centrifuge using the special R.I.A. rotor.

Reagents

Stock molybdate reagent

To a 10 litre volumetric flask, containing about 7 litres of water, add:-

1 litre 5M sulphuric acid (prepared by diluting 98% sulphuric acid)

24,0 g ammonium molybdate [$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$]

1,0 g antimony potassium tartrate [$\text{KSbO}_3\cdot\text{C}_4\text{H}_4\text{O}_6$]

7,0 g sodium lauryl sulphate [$\text{C}_{12}\text{N}_{25}\text{NaO}_4\text{S}$]

Dilute to mark with water, shaking to dissolve reagents.

*Post graduate material submitted to the University of Natal Applied Chemistry Department.

Mixed reagent

Dissolve 1,50 g ascorbic acid in 500 ml of stock molybdate reagent. Prepare daily sufficient of this mixed reagent for a batch of samples.

Calibration Standards

Dissolve 2,191 g dried potassium dihydrogen orthophosphate in 0,01M sulphuric acid to make 1 litre. Dilute 10 ml of this solution to 500 ml with 0,01M sulphuric acid to give 10 mg P/l solution. Further dilute this solution to give equivalent soil phosphorus levels in the range 0 to 100 ppm soil P.

Analytical Procedure

Manual complex preparation

Add 3,33 ml mixed reagent to 5 ml of calibration standard or sample in a 100 ml conical flask. Mix and allow to stand for 10 minutes. Transfer a portion of sample to an LKB cuvette and measure the absorbance of the colour complex as described below.

Automatic complex preparation

Use the LKB 2071 sample processor to dilute the standards and soil extracts with the mixed reagent, using LKB cuvettes to collect the colour complex. Instrument settings and conditions are shown in Figure 1. Allow to stand for 10 minutes after the last rack has been processed. Read the complex absorbance values using the LKB 7400 calculating absorptiometer with a 695 nm interference filter. Use a 30 ppm soil P standard to factorise the instrument.

Special care must be taken in the preparation of calibration standards and reagent blanks to eliminate the adverse effects of sample carryover. Calibration standards must be prepared in duplicate and the second sample used. Reagent blanks should be prepared in isolation and inserted into the appropriate position in an LKB rack before taking measurements.

Measures to minimise the effects of sample carryover

Examine the printout obtained using the above procedure and identify the following:

1. sample values (x) less than 30 ppm P (70 kg/ha P) for which the preceding sample has a value $> x + 30$ ppm P ($> x + 70$ kg/ha P)
2. sample values (y) between 31 and 66 ppm P (70 and 150 kg/ha P) for which the preceding sample has a value $> y + 70$ ppm P ($> y + 150$ kg/ha P)
3. the two sample values immediately following a value of > 250 ppm P (> 600 kg/ha P)

Discard these results, re-prepare and measure the colour complexes. Samples with values less than 30 ppm should be

processed first followed by those with values between 31 and 66 ppm.

Development of the Method

A manual method was devised from the recommended procedure of Murphy and Riley¹⁰ by diluting the reagent to give a sample to diluent volume ratio of 3:2, ensuring that the final solution reactant concentrations remained unchanged. This manual procedure was used to evaluate the colour complex so that results would not be confounded by possible carryover effects from the use of the LKB sample processor.

The wavelength of the colour complex side peak was found to lie between 690-720 nm from a plot of absorbance versus wavelength (Figure 2) produced using a Beckman model 24S spectrophotometer and chart recorder. Subsequent measurements were taken using a 695 nm interference filter (15 nm band pass) with the LKB 7400 calculating absorptiometer.

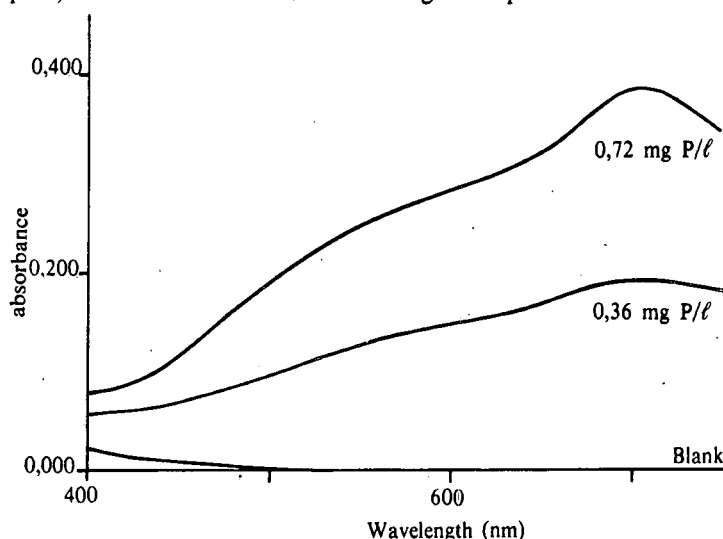


Figure 2: Absorbance spectra for Murphy and Riley's method

Colour complex stability was evaluated by taking repeated absorbance measurements using complexes prepared over a range of phosphorus concentrations. The results (Figure 3) confirmed those of Murphy and Riley¹⁰ and showed that the complex was stable for at least 22 hours.

Using simulated soil extracts, the effect of varying reactant concentration on complex formation and stability was also investigated. This was done to gain some idea of the "ruggedness" of the method and to check that reaction conditions were optimum. A full factorial experiment, in which all possible permutations of selected reactant concentrations are examined, was considered unnecessary. The less time-consuming method of successive approximation was therefore used. Initially the con-

PUMP D			PUMP C			PUMP B			PUMP A			RACKS			S
R	1	2	R	1	2	R	1	2	R	1	2	R	R	R	
							●			●				●	●
						D/60-900 µl			S/60-900 µl			Pump type			
						600			900			Volume µl			
						mixed reagent			sample			REAGENT			

Figure 1: Sample processor programme for soil phosphorus determination

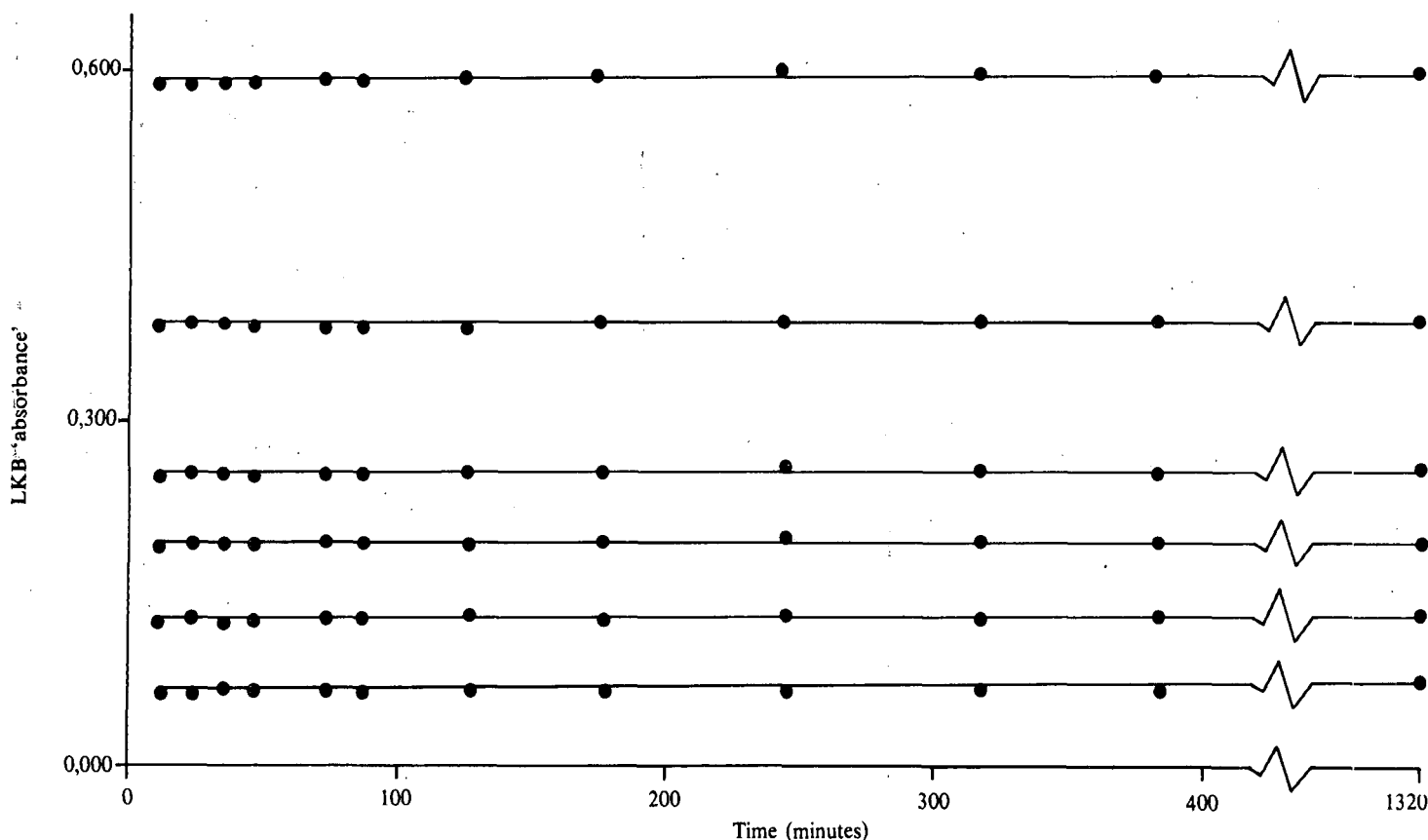


Figure 3: Colour complex stability (Murphy and Riley's method)

centration of three reactants was kept at that suggested by Murphy and Riley¹⁰ and a fourth was varied, the experiment being repeated for each of the reactants.

Final solution concentrations of sulphuric acid and ammonium molybdate could be varied by at least $\pm 10\%$ of the recommended rate with no adverse effect. Increases in ascorbic acid concentration to three times (x3) the recommended rate had no adverse effects but its reduction by a half (x0,5) caused colour complex instability.

The effective working range over which Beer's Law was obeyed could be increased by raising the antimony potassium tartrate concentration. Complexes containing 22 mg/l antimony potassium tartrate (as recommended by Murphy and Riley¹⁰) obeyed Beer's Law up to 1 mg P/l final solution (see Figure 4). The range of P concentration over which Beer's Law was obeyed could be increased to 1,2 mg P/l (100 ppm P in soil) by raising the antimony potassium tartrate concentration to an estimated 25 mg/l. Above 1,2 mg P/l Beer's Law was no longer obeyed irrespective of antimony concentration. Complexes with final solution concentrations of up to 1,8 mg P/l (150 ppm P in soil) deviated least from Beer's Law, when the concentration of antimony potassium tartrate was greater than an estimated 38 mg/l.

Increasing the antimony concentration, when sufficient was present to give maximum absorbance at a particular P concentration, had no effect on relative complex stability. Complexes with final solution concentrations of up to 1,44 mg P/l (120 ppm P in soil) were reasonably stable for 24 hours at all the antimony concentrations tested. Absorbance values for all the complexes containing 1,8 mg P/l declined significantly on storage for 24 hours when sufficient antimony (III) was present to ensure maximum initial colour development. In these cases a blue precipitate was formed in the cuvette indicating possibly that the reduced molybdoantimonylphosphate complex was not very soluble.

The procedure was then adapted for use with the LKB 2071

sample processor. It was calculated that up to equal quantities of sample and diluent could be used without a reduction in sensitivity compared to the manual Dickman and Bray procedure. Improvements in sensitivity could be made by reducing the diluent volume until the effective working range became a limiting factor at a sample to diluent ratio of 3:1. At this ratio complexes obeyed Beer's Law up to the equivalent of 80 ppm soil P.

The volume of sample was reduced to 900 μ l which just filled the sampler probe and connecting pipe. Where necessary the concentration of mixed reagent was adjusted to retain final solution reagent concentrations unchanged. Complexes were then prepared with the LKB sample processor and total carryover determined using the method described by Broughton *et al*², except that sample values were averaged to reduce the effect of instrument error on the result.

Values obtained at the 1:1, 3:2 and 3:1 sample to diluent ratios are shown in Table 1. A wide range of soil 'available' phosphorus values are possible and since total carryover is a function of the difference between values for adjacent samples, the levels of carryover experienced could have serious consequences.

Grigg⁸ considers that a relative standard deviation of less than eight percent in the analytical steps of soil analysis to be very satisfactory and maintains that increased accuracy is not warranted in view of the variability in field sampling. Using this as a criterion to evaluate carryover, it is clear that the reduction in the result for a high P sample caused by a preceding sample with a low P content would have no serious consequences. The converse is not true, however, and values in the important range of samples containing less than 10 ppm soil P could be seriously affected.

Surfactants have been widely used in continuous flow analysis (Colwell⁵, Thomas *et al*¹¹, Wall and Gehrke¹²) to reduce carryover and ensure good sample bubble formation. No information regarding the possible beneficial effects of using a surfactant with the LKB sample processor was available. Table 1

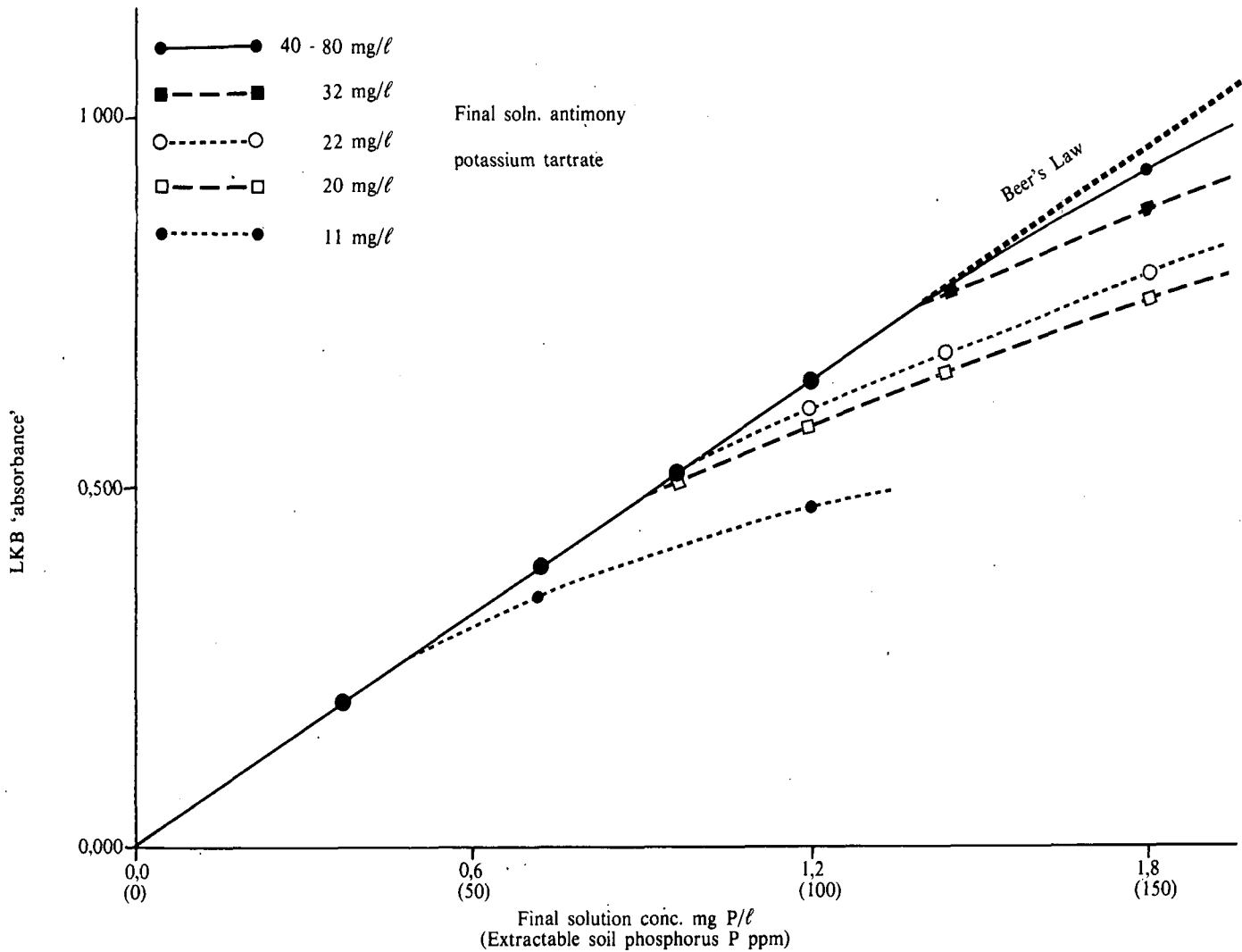


Figure 4: Effect of varying antimony concentration on Beer's Law plot

also shows the effect of incorporating 0,1% sodium lauryl sulphate (a compatible surfactant) into the diluent. Total carryover was significantly increased in all cases by the use of a surfactant. However several overriding beneficial features were noted. The use of a surfactant considerably improved the physical operation of the instrument by eliminating cuvette air bubbles and marginally reducing splashing. Sample pumps were also more easily primed due to lowering of surface tension effects. In view of these factors a surfactant was considered desirable despite the increased carryover resulting from its use.

In 1970 a survey of routine samples results (Anon¹) revealed that almost 60% of soils had 'available' phosphorus values less than 30 ppm P. A scheme was therefore devised to identify samples where unacceptable levels of carryover were likely to be found. These samples could then be re-analysed in isolation. Since 60% of samples were likely to have similar values, the extra work resulting from this procedure could be kept to a minimum. The compromise situation where some of the increased sensitivity was used to raise the diluent volume was con-

sidered on balance to be most suitable. The 3:2 sample to diluent ratio was therefore chosen for use with the automated procedure.

Routine Testing

Reproducibility of standards

Similar LKB 'absorbance' values (see Table 2) were obtained irrespective of the method of preparation. Relative standard deviations for complexes prepared with the sample processor are on balance slightly lower, indicating equivalent or greater precision than that in the manual method of preparation and in both cases values are very reproducible.

Comparative testing

An extensive comparative testing programme was undertaken since external reference material to establish the accuracy of the new method was not available. The objects of the work were to check that bias was not introduced with a change in colorimetric

TABLE I
Mean total carryover obtained using 900 μ l sample with different diluent volumes

Diluent Volume μ l	Sample to diluent volume ratio	Without surfactant		0,1% sodium lauryl sulphate	
		Mean total carryover	Standard deviation	Mean total carryover	Standard deviation
900	1:1	0,020	$\pm 0,003$	0,027	0,001
600	3:2	0,027	$\pm 0,004$	0,041	$\pm 0,003$
300	3:1	0,041	$\pm 0,005$	0,062	$\pm 0,001$

TABLE 2
Reproducibility of complexes prepared using the manual and automated procedures

Soil level P ppm	Final solution P mg/l	Manual procedure		Automated procedure	
		Mean LKB 'absorbance'	Relative standard deviation%	Mean LKB 'absorbance'	Relative standard deviation%
10	0,12	0,068	7,7	0,067	6,1
20	0,24	0,132	3,7	0,130	1,8
30	0,36	0,192	2,2	0,192	1,6
40	0,48	0,258	1,9	0,256	1,5
60	0,72	0,385	1,2	0,381	1,4

procedure and to examine the effectiveness of the controls to limit the effects of carryover during automated sample preparation.

Routine extracts (113 samples) were split and portions analysed by the existing and improved manual procedures except that the calculating absorptiometer was used to make both measurements. There were no significant differences, at the five percent confidence level, between values obtained by the two methods.

To test the 'ruggedness' of automatic sample preparation, 10 percent of routine sample extracts over a period of a month (295 samples) were analysed using the proposed manual and automatic methods of complex preparation. Only 13 of these samples differed by more than the 99 percent confidence limits calculated from the relative standard deviation values given in Table 2. Repeat analysis showed that four discrepancies were due to manual preparation, three due to automatic preparation and in six cases the cause was not established. From this it would appear that the effects of carryover have been effectively controlled and the use of the sample processor does not increase the number of incorrect results. Large errors can be caused by the occasional container contaminated with orthophosphate and problems from this source were expected to increase with a reduction in the scale of analytical operations. The results clearly indicate that random sample contamination is not a serious problem when the sample processor is used, and values are as reliable as those obtained with the improved manual procedure.

Advantages of the new method

Results obtained during the routine testing of the improved procedure for soil phosphorus clearly indicate its suitability for general use by the FAS laboratory. Both the manual and automated procedures are sufficiently robust and give unbiased results. Relative standard deviations of about two percent were obtained when the calibration standards, used to factorise the calculating absorptiometer, were repeatedly prepared and measured. The stable colour complex means that large batches of samples can be simultaneously prepared. Work output was increased with the use of the automatic soil phosphorus method.

A batch of 200 soil extracts can now be prepared and measured by one laboratory assistant in 80 minutes, compared with the 8 man-hours previously required. One disadvantage of the automated procedure is that a limited number of samples require re-analysis in isolation to eliminate biased results caused by the adverse effects of sample carryover.

Acknowledgements

Thanks are due to Professor D.E.A. Williams-Wynn who supervised this work, Mr. M. G. Murdoch for his advice on statistical analysis and Dr. R. A. Wood for his careful reading and constructive criticism of the manuscript.

REFERENCES

1. Anon. (1970). S.A. Sugar Assn. Experiment Station Annual report 1969-70 : 22-23.
2. Broughton, P.M.G., Buttolph, M.A., Gowenlock, A.H., Neill, D.W., and Skentelbery, R.G. (1969). Recommended scheme for the evaluation of instruments for automatic analysis in the clinical biochemistry laboratory. *J Clin Path* 22 : 278-284.
3. Burrows, J.R. (1977). An improved method for the determination of nitrogen in cane leaves. *SASTA Proc* 51 : 68-71.
4. Burrows, J.R. and Meyer, J.H. (1976). Semi-automation with references to the determination of aluminium and phosphorus in soil and cane leaves. *SASTA Proc* 50 : 114-117.
5. Colwell, J.D. (1965). An automatic procedure for the determination of phosphorus in sodium hydrogen carbonate extracts of soils. *Chem and Ind* 21 : 893-895.
6. Dickman, S.R. and Bray, R.H. (1940). Colorimetric determination of phosphate. *Ind. Eng. Chem Anal Ed* 12 : 665-668.
7. du Toit, J.L. (1959). Recent advances in the nutrition of sugarcane in South Africa. *ISSCT Proc* 10 : 432-441.
8. Grigg, J.L. (1975). Determination of phosphate in soil extracts by automatic colorimetric analysis. *Comm Soil Sci and Plant Anal* 6 : 95-112.
9. L.K.B. Ultralab system application notes (1973) AN 94. File 1.
10. Murphy, J. and Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters *Anal Chim Acta* 27 : 31-36.
11. Thomas, R.L., Sheard, R.W., and Moyer, J.R. (1967). Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. *Agron J* 59 : 240-243.
12. Wall, L.L. and Gehrke, G.W. (1975). An automated total protein nitrogen method. *J.A.O.A.C.* 58 : 1221-1226.