


CEFAS BIOGEOCHEMICAL PROCESSES STANDARD OPERATING PROCEDURE (SOP)	
S.O.P. No. EQ-BGC-007B	Issue: 1 Page 1 of: 7

The simultaneous determination of nitrate, nitrite, phosphate, silicate and ammonia using continuous flow analysis

Production Summary

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Introduction

Manual colorimetric techniques for the determination of nutrients in water samples is very well established but is time consuming and subject to errors from varying conditions. Continuous flow analysis (CFA) enables large numbers of samples to be analysed within strictly prescribed and maintained operating conditions and computerised control also allows simple data acquisition and calculation and presentation of results. A good understanding of the chemical parameters allows them to be controlled and optimised to achieve accurate results at less than $0.5 \mu\text{mol l}^{-1}$. The procedure follows Kirkwood (1996).

Scope


This SOP describes the procedure for the determination of the named nutrients using continuous flow analysis. It does not include the collection and preparation of samples for nutrient analysis (EQ-BGC-007), or the preparation of reagents required (EQ-BGC-007A).

Training

Operator must have been trained thoroughly in GLP (good laboratory practice) and have read procedures COSHH/LOW/EQBGC/NG003, COSHH/LOW/EQBGC/NG005, COSHH/LOW/EQBGC/NG006, COSHH/LOW/EQBGC/NG007. It is advisable for the operator to read this procedure thoroughly before starting the process and run through initial analytical runs with an experienced operator. The operator should also refer to the manuals supplied with the analyser.

Apparatus

1. Autosampler (1 x SA1000)
2. Skalar module holder with analytical modules for nitrite, nitrate, phosphate, silicate and ammonia and rinsing valves
3. digital interface
4. laptop computer with Flow Access software and printer
5. two water circulation baths
6. 10-100 μl volumetric pipette
7. 100-1000 μl volumetric pipette
8. 1ml disposable plastic pipette
9. 50ml volumetric flask
10. 100ml volumetric flask
11. 250ml LDP bottles containing low nutrient seawater
12. ultra pure water (UPW)
13. 20ml and 75ml polycarbonate pots with lids
- 14.

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Procedure

Preparation of instrumentation and analysis of samples

1. Make sure that the black 2.5L bottle containing the rinsing liquid is filled with UPW.
2. Place bottles containing reagents in the space provided on the left side of the instrument case.
3. Place reagent lines in the bottles and depress the relevant buttons on the rinsing valve units to allow reagents to be taken up from the bottles and into the instrument for the nutrients which are of interest. If analysing for nitrate, open the cadmium column to allow the flow of nutrients through it.
4. Turn the power on to the following pieces of instrumentation in the order given; autosampler, Skalar module holder, water baths and computer.
5. Allow computer to boot-up and then switch on the digital interface
6. Open the Flow Access software and click on *Active System* and press *Enter* to open the CEFAS active system. Check to see that the lamps in the flow cells light up.
7. The main analysis window will appear with shortcut buttons and drop-down menus at the top of the screen. Underneath is the layout of the CEFAS configured system with boxes next to each of the nutrients.
8. Use the mouse to click on the tick button and tick the boxes next to the nutrients you wish to analyse for.
9. Use the mouse to click on the table shortcut button. Press *Enter* to edit the current table. (Note: it is possible to save every table as a different file but this takes up a lot of computer memory and is unnecessary as the table used for a particular run will be saved with the results of the run, even if the table is subsequently modified for a later run).
10. Edit the table to include the standards you require and enter the sample details of the unknowns. The standard format of a run table is as follows:

Tracer

Drift

Wash

standards

drift

wash

10 samples (unknowns)

drift


wash

10 samples (unknowns)


drift

wash

11. Save changes to the table and print a copy to assist with loading samples in the correct order for analysis. Exit to the main analysis window.


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12. Move the number wheels on the turntable to show the total number of samples to be analysed in the run.
13. Use the mouse to click on the shortcut button for the real time data. The real time window will appear and individual nutrients can be viewed using the shortcut buttons at the bottom of the window.
14. Allow the baselines of all nutrients of interest to stabilise whilst preparing calibration standards.
15. Prepare the calibration standards as detailed in Appendix 1. If analysing seawater or estuarine samples, prepare calibration standards using low nutrient seawater. If analysing freshwater samples, prepare calibration standards using UPW contained in the 1L poly bottles (ie. do not use UPW from the 25L carbuoy of UPW for general lab use to prepare the calibration standards).
16. After preparation, keep standards in a fridge until use. Fresh standards must be prepared on a daily basis – do not use standards made a previous day.
17. Once standards are prepared click on the *Start* shortcut button. Enter your name in the box which appears and press *Enter*.
18. The next box to appear will ask for a filename to save the run under. Enter the filename in the format dd_mm_yy. If there are a large number of analytical runs to be carried out from the same piece of work create a new folder titled with the contract name in which to save all the analytical runs.
19. Press *Enter* and an information box will appear informing you that the analytical run is starting. Place the first standard on the turntable and once the information box has disappeared press down the *Start* button on the turntable to start the analytical run.
20. If analysing for ammonia it is best to place each standard/sample individually on the turntable to minimise the time the sample is in contact with air. This will minimise the amount of ammonia which can be absorbed from the air by the sample. As the sample uptake time is 90s and the wash time is 120s, a new standard/sample will need to be placed on the turntable every 3.5 minutes.
21. If ammonia is not to be analysed, samples may be loaded into the sample turntable and left to run.
22. Use the mouse to click on the real time shortcut. This will open the real time window and the operator must regularly check this window to verify that the peak shapes and labelling are satisfactory. If there are any problems these can be identified early and the run can be stopped if necessary rather than identifying problems at the end of the run and wasting a whole analytical run.
23. Another useful window to open is the *Results*. Regular checking of this window will also enable problems to be identified early.
24. At the end of the analytical run, the real time and results windows will automatically close and the screen will return to the main analysis screen.
25. Once returned to the main analysis screen, close the cadmium column if it was used and turn the power off to the heater baths. Take the reagent lines out of the reagent bottles, remove the glass weights and rinse and place in pot with UPW. Place reagent lines in UPW bottles provided solely for this purpose and allow UPW to rinse through the system for a minimum of 15 minutes. Once the system has been rinsed switch off the power to all units.

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Data processing

1. Click on the drop down *File* menu in the *Analysis* screen and select *Post Analysis*.
2. A box will appear to prompt you which file you wish to open. Select the file from the list and click *Open*.
3. The *Post analysis* screen will appear, with a similar layout to the *Analysis* screen.
4. If there are any adjustments needed to the run table make these first by editing the table.
5. Open the real time window to review peak identification. Using the *modify* button, adjust any peak markings which are incorrect then close this window.
6. Open the results window. Review the regression coefficients for each chemistry in turn. The standards to be included in the regression may be edited if necessary by using the *edit method* facility.
7. The layout of the results may be altered using *options, format results* which allows the operator to chose which data is presented.
8. Once the data editing is complete print out the calibration graphs for each of the chemistries and the results table.
9. Copy the runfile onto the *data transfer* CD and then copy this from the CD into the *scalar archive* folder. Update SOP-EQ-BGC-002A with the details of the runfile.
10. In the *Nutrients* directory, locate the Excel results spreadsheet in which to enter the nutrient results. SOP-EQ-BGC-002 contains a list of all Excel results files and which contract and piece of work they refer to.
11. Open the relevant Excel results spreadsheet and enter the results from the run.

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Appendix 1

Analyte		std 1	std 2	std 3	std 4	std 5	std 6	std 7	std 8	std 9	std 10	std 12	OSIL 3	OSIL 6
Nitrate	to make 100ml of working standard					200					12		1440	720
	to make 50ml of working standard	250	200	150	125	100	75	50	25	12		LNSW	720	360
	concentration in umol/l	24.00	19.20	14.40	12.00	9.60	7.20	4.80	2.40	1.15	0.58		14.40	7.20
Nitrite	to make 100ml of working standard					500					31		3000	1500
	to make 50ml of working standard	625	500	375	315	250	190	125	62	31		LNSW	1500	760
	concentration in umol/l	5.00	4.00	3.00	2.50	2.00	1.52	1.00	0.50	0.25	0.12		3.00	1.52
Phosphate	to make 100ml of working standard					500					31		3000	1500
	to make 50ml of working standard	625	500	375	315	250	190	125	62	31		LNSW	1500	760
	concentration in umol/l	5.00	4.00	3.00	2.50	2.00	1.52	1.00	0.50	0.25	0.12		3.00	1.52
Silicate	to make 100ml of working standard					800					50			
	to make 50ml of working standard	1000	800	600	500	400	300	200	100	50		LNSW	600	300
	concentration in umol/l	20.00	16.00	12.00	10.00	8.00	6.00	4.00	2.00	1.00	0.50		12.00	6.00
Ammonia	to make 100ml of working standard					400					25		60	30
	to make 50ml of working standard	500	400	300	250	200	150	100	50	25		LNSW	30	15
	concentration in umol/l	10.00	8.00	6.00	5.00	4.00	3.00	2.00	1.00	0.50	0.25		6.00	3.00
TOXN	concentration in umol/l	29.00	23.20	17.40	14.52	11.60	8.72	5.80	2.90	1.40	0.70		17.40	8.72

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