1. Introduction and Scope

This method describes the procedure to determine nutrients (TOxN, NO_2, NH_3 [QuAAtro only], PO_4 and SiO_2) in estuarine and sea waters, using the Bran and Luebbe AA3 and QuAAtro autoanalysers, on site at the Marine Laboratory, Aberdeen and in a containerised laboratory.

The method is used for the analysis of samples from FRS groups and external customers for various purposes. Fitness for purpose of the method in all cases is confirmed through contract review.

The range of the method is as follows:

Range µM					
AA3			QuAAtro		
	Laboratory	Containerised Lab		Laboratory	Containerised Lab
PO_4	0.01-5	0.01-5	NH_3	0.027-10	0.088-10
TOxN	0.01-11.75	0.01-23.5	PO_4	0.006-5	0.009-5
SiO_2	0.01-20	0.02-20	TOxN	0.007-23.5	0.009-23.5
NO_2	0.01-10	0.02-10	SiO_2	0.006-20	0.009-20
			NO_2	0.002-10	0.01-10

2. Principle of the Method

The continuous flow analysis (CFA) system consists of an autosampler, peristaltic pump, a chemistry manifold containing all the components for a specific analysis i.e. heating baths, mixing coils and reduction columns, and a detector and computer to handle the data output.

Sample and reagents are pumped continuously through the chemistry manifold. Air bubbles are added to separate each sample from the next as it passes through the tubing, this helps to minimise inter-sample dispersion.

The sample and reagents are mixed in each liquid segment by pumping them through glass mixing coils where gravity and the internal flow of the liquid assist in the mixing. Longer coils and heating baths can be used to improve the reaction time. A reduction column is placed in the TOxN sample line to reduce the nitrate (NO_3) component of the TOxN to nitrite (NO_2).

The sample stream is passed through a dual beam digital colorimeter, with an LED light source. LEDs are used as they produce light at a stable wavelength even in environments with a lot of vibration such as on a ship. The sample

absorbance is measured by a colorimeter and the data recorded on a computer.

2.1 Total Oxidised Nitrogen

Nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion reacts with sulphanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-napthylethylene diamine hydrochloride to form a purple azo dye. The colour intensity is measured at 520 nm and quantified with standards.

2.2 Nitrite

The nitrite ion reacts with sulphanilamide under acidic conditions to form a diazo compound. This compound then couples with N-$_1$-napthylethylene diamine hydrochloride to form a purple azo dye.

The absorption is measured at 520 nm and is quantified using standard solutions. Nitrite concentrations usually represent only a proportion of the total oxidised nitrogen (NO_3/NO_2) found in oceanic water and may be ignored. Nitrite may however be present at significant concentrations in some estuaries (e.g. Forth).

2.3 Phosphate

This method is based on the colorimetric method in which a blue colour is formed by the reaction of orthophosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at a pH<1. The reduced blue phospho-molybdenum complex is read at 880 nm and quantified using standards.

2.4 Silicate

This method is based on the reaction of silico-molybdate in acidic conditions to 'molybdenum blue' by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to minimise interference from phosphates. The colour intensity is measured at 820 nm and quantified using standards.

2.5 Ammonia (QuAAtro only)

This method uses the Berthelot reaction, in which ammonium ions react with phenate and free chlorine to form a blue-green coloured complex. Sodium nitroprusside is used to enhance the sensitivity. It is measured at 630nm and quantified using standards.

3. Reference Material

See SOP 0725

4. Reagents

All chemicals are of Analar grade unless otherwise stated. For analysis in a containerised laboratory, dry chemicals are pre-weighed into clearly labelled containers at the MLA. All weights and equipment used are recorded in the

logbook kept in room 501b.

Reagents are prepared and checked as per SOP 0720

Standards are prepared and checked as per SOP 0721

5. Equipment

Bran & Luebbe Autoanalyser 3 (AA3) – AA3 Compact sampler (EN698)
AA3 Pump (EN699)
AA3 Chemistry module (EN696)
AA3 Digital colorimeter (EN711)
PC with AACE software

QuAAtro Autoanalyser - QuAAtro Analyser (EN1318)
Digital Colorimeter (EN1319)
Autosampler (EN1320)
PC with AACE software

6. Environmental Control

Nutrient analysis requires a stable temperature, avoiding contamination of samples and reagents, e.g. contact with fingers, dirty equipment, smoking etc. disposable gloves should be worn when handling chemicals. These should be Duratouch disposable vinyl gloves. Beakers, measuring cylinders and volumetric flasks are kept clean using Decon (phosphate free detergent obtained from Norlab Instruments Ltd) (refer to SOP0220). Addition of sulphuric acid must be carried out in a fume cupboard wearing protective clothing.

7. Interferences

Samples that are likely to contain high particulates e.g. water from around fish farms or estuarine water - must be filtered by the client before accepted for analysis.

8. Sampling and Sample Preparation

Samples are logged into the laboratory according to SOP 0060.

Analysis at MLA

Samples for TOxN and Phosphate analysis are taken in the field in glass bottles and then frozen. The samples must be stored in a clean freezer used only for the storage of nutrient samples. Anticipate when the samples will be required for analysis and remove from the freezer the night before to thaw.

Samples for silicate analysis are taken in the field in plastic bottles and preferably analysed as soon as possible after sampling. If this is not possible the samples must be stored in the cool and dark (e.g. refrigerator). Remove samples from the fridge and allow them to come to room temperature on the bench, ensure the bottles are not in direct sunlight. Samples kept in plastic

bottles can be analysed for any determinant if required, as a check on results from the samples stored in glass bottles.

Analysis in a containerised laboratory
Samples which are collected for analysis in a containerised laboratory are collected in plastic bottles, and stored in the dark on ice until analysis. Remove the samples from the ice and allow them to come to room temperature on the bench before analysis. All samples should be analysed within 10 hrs of sampling (Grassholf et al), note on record sheet B 146.

Samples will be disposed of according to SOP 0060.

9. Analytical Procedure

9.1 Start up procedure as per SOP 0725

9.2 Shut-down procedure as per SOP 0725

9.3 Checks and maintenance.
Checks following maintenance and reagent preparation are as per SOP 0725 section 9.2.

9.4 Calibration and quality control

Calibration standards (highest to lowest) are run at the beginning of each run. After approx. 25 samples and at the end of each analysis batch a drift cup is run (top standard). With each analysis set a reference material should be randomly analysed, at least once every 25 samples.

10. Calculation of Results- See SOP 716 section 10.

11. Mthod Validation

Validation raw data is maintained under nts5/ukas/method validation/.

Summary method performance information is maintained in B045

12. Reports
Record the concentration of the reference material on B 144. Printouts are used as records in the archive. Reports are issued according to SOP 1350.

13. Safety
Wear laboratory protective equipment. Wear gloves when handling chemicals. Use of concentrated acids must be carried out in a fume cupboard.
See SOP 720, SOP 721 and SOP 725 for risk assessments.

14 Literature References

Bran & Luebbe Autoanalyser 3 operation manual
Bran & Luebbe AACE software manual
Bran & Luebbe Continuous flow applications
Bran & Luebbe compact sampler operation manual
Bran & Luebbe XYZ sampler operation manual
Bran & Luebbe QuAAtro Autoanalyser operation manual

15. Uncertainty of Measurement:

Uncertainty values are maintained under B045

Sources of uncertainty:

Sampling: Samples are analysed and results reported on the samples as received – outwith uncertainty calculations;

Sub-sampling: Seawater sample is considered homogeneous so sub-sampling does not contribute to the error – negligible contribution to uncertainty;

Storage conditions: Samples for TOxN, NO_2, NH_3 and PO_4 analysis are stored in glass bottles and frozen (not for longer than two months). Samples for SiO_2 analysis are stored in plastic bottles and kept refrigerated (not for longer than 2 months) – negligible contribution to uncertainty.

Reagent purity: All reagents used are at least Analar quality, considered sufficient – uncertainty accounted for in validation data.

Instrument effects: Carry-over minimised by running a wash sample between samples and the software performs a carryover correction. Typical standard curve r-value 0.9995. Uncertainty accounted for in validation data

Efficiency of cadmium column effect – only for TOxN Channel

Cadmium column is checked for efficiency of above 95% on the day of each run. Uncertainty accounted for in validation data and control chart data.

Weight: Tolerance of balance/decimal places – balances check weight tolerance generally <1%. 1-2 decimal places used, sufficient for accuracy required. Uncertainty accounted for in validation data

Volume: Pipettes and dispensers used in preparation of reagents – reagents prepared are to excess, associated error not critical, and always <10%. Pipettes used for calibration standards calibrated to <1%. Uncertainty accounted for in validation data

Time: Not applicable

Computational Effects: Concentrations are calculated by instrument software. Manual check of calculation has been carried out and acceptable – negligible contribution to uncertainty.

Blank Correction: Standards are made up using Low nutrient sea water. Low nutrient sea water is run with the set of standards at the start of each run then the concentration in the sea water added to the concentration of each standard to give the true concentration of each standard. Uncertainty is accounted for in validation data

Environment conditions: Contamination is minimised by the use of a dedicated

laboratory and equipment. Gross temperature changes are avoided by the temperature controlled make-up air – laboratory only. The QuAAtro has a thermostatically controlled chemistry manifold to minimise the effects of changes in the air temperature. Uncertainty accounted for in validation data

Operator Effects: All measurement methods are described in fully documented standard operating procedures to limit inconsistencies between operators. Only trained personnel may perform method unsupervised. Variations between operators are accounted for by control chart data. Uncertainty accounted for in validation data.

Matrix Effects: Method is matrix-matched specifically for seawater. Standards are prepared in Low Nutrient Seawater. Uncertainty accounted for in validation data

Random effects: These will be accounted for by validation and control chart data.