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Determination of total nitrogen in food, environmental and other samples by ion chromatography after Kjeldahl digestion

PETER E. JACKSON*[a], JIM KROL and ALLAN L. HECKENBERG
Waters Chromatography Division, Millipore Corporation, 34 Maple Street, Milford, MA 01757 (U.S.A.)
and
MARINA MIENTJES and WIM STAAL
Millipore BV, Penningweg 33, Postbus 166, 4870 AD Etten-Leur (The Netherlands)

ABSTRACT

Total nitrogen in food, environmental and many other matrices is usually determined by Kjeldahl digestion–distillation followed by titration. The use of ion chromatography to determine total nitrogen as ammonium ion after sample digestion significantly improves the speed of the analysis compared with the conventional method. A poly(styrene–divinylbenzene) cation-exchange column with dilute nitric acid as eluent and indirect conductivity detection was used for the ion chromatographic determination of ammonium in the presence of the elevated levels of sulfuric acid found in the digested sample. The peak-area precision for ten replicate injections of an ammonium standard was 0.6% [relative standard deviation (R.S.D.)] and the retention time precision was 0.3% (R.S.D.). The determination of ammonium was linear from 15 ppb (10^9) up to 25 ppm. The results obtained by the ion chromatographic method were compared with those for the conventional distillation and titration approach for the determination of total nitrogen in animal feeds. The application of the ion chromatographic method is also demonstrated for a variety of other sample matrices.

INTRODUCTION

The determination of total nitrogen in food, environmental and many other sample types is typically carried out using Kjeldahl digestion–distillation followed by titration [1,2]. This well established procedure involves digesting the sample in an acid mixture in the presence of a catalyst, distillation of ammonia in the digest into a dilute acid solution, followed by back-titration with base. Several alternatives to the classical approach for total nitrogen have recently been suggested, including the use of microwave digestion [3] and combustion methods [4,5].

Ion chromatography (IC) has become a well established technique for the determination of ammonium ion [6,7]. The use of ion chromatography to determine total nitrogen as ammonium ion after Kjeldahl digestion can significantly improve the speed of the analysis compared with the conventional method by eliminating the need

[a] Present address: Millipore Pty. Ltd., Private Bag 18, Lane Cove, NSW 2066, Australia.

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for the distillation step. As only microliter volumes of sample are required for analysis, the weight of sample required for the digestion can be reduced, minimizing the amount of catalyst [*e.g.*, mercury(II) oxide or sulfate] that needs to be used, and hence also disposed of. In this work, the use of IC for the determination of total nitrogen in grain samples, organic compounds and sewage sludges after Kjeldahl digestion was investigated. The selection of appropriate analytical conditions is discussed and the results obtained by the digestion–IC method are compared with those obtained by the classical digestion–distillation technique.

EXPERIMENTAL

Instrumentation

The liquid chromatograph consisted of a Waters (Milford, MA, U.S.A.) Model 510 pump, a U6K injector, a Model 431 conductivity detector and a Waters Model 820 data station. The analytical column used was either a Waters IC-Pak Cation (50 × 4.6 mm I.D.) or a Waters Protein-Pak SP-5PW (75 × 4.6 mm I.D.). The eluents used were 2.0 m*M* nitric acid at 1.2 ml/min or 25 m*M* nitric acid–5% acetone at 2.4 ml/min with the IC-Pak Cation or the Protein-Pak SP-5PW column, respectively. The eluents were prepared daily, filtered and degassed with a Waters solvent clarification kit before use.

Reagents

Water (18 MΩ) purified using a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.) was used to prepare all solutions. Ultrex nitric acid and acetone and analytical-reagents grade chloride salts used for the preparation of the cation standards were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.).

Kjeldahl digestion

Two different sample digestion procedures were carried out using standard Kjeldahl methods. Organic compounds and sewage sludges were digested using a potassium sulfate–sulfuric acid mixture with a mercury(II) oxide [or mercury(II) sulfate] catalyst [1] and animal feed (grain) samples were digested using an acetic acid–sulfuric acid mixture with hydrogen peroxide as the catalyst [2].

RESULTS AND DISCUSSION

Selection of chromatographic conditions

The chromatographic conditions selected for the determination of ammonium ion depended on the actual digestion precedure employed. The determination of ammonium ion in the grain samples digested using the acetic acid–sulfuric acid procedure was relatively straightforward and could be carried out, after dilution, using 2 m*M* nitric acid as eluent, a low-capacity cation-exchange column (IC-Pak Cation) and indirect conductivity detection. The determination of low ppm levels of ammonium in samples digested using the potassium sulfate–sulfuric acid mixture could not be accomplished using the IC-Pak Cation column owing to the presence of *ca.* 20 000 ppm of potassium in the final digest. This analysis was carried out using a significantly higher cation-exchange capacity column (Protein-Pak SP-5PW) to ensure resolu-

tion of the ammonium peak from the adjacent potassium peak. This column was also suitable for the determination of ammonium in the acetic acid–sulfuric acid–digested samples. The solution obtained from either digestion procedure typically contained ammonium in the range 100–500 ppm and was very acidic (pH < 1). This solution was simply diluted 100–1000-fold before injection into the chromatograph; no other sample pretreatment was necessary.

The retention time and peak-area precision, detection limits and linearity for the ion chromatographic determination of ammonium ion were then established using the IC-Pak Cation column. The retention time precision of ten replicate injections of a 5 ppm ammonium standard was 0.3% [relative standard deviation (R.S.D.)] and the peak-area precision from the ten replicate injections was 0.6% (R.S.D.). The detection limit (three times the signal-to-noise ratio) for ammonium was 15.7 ppb (10^9) using a 100-μl injection and the determination of ammonium was linear up to 25 ppm. A chromatogram of a cation standard containing lithium, sodium, ammonium and potassium obtained using the IC-Pak Cation column and 2 m*M* nitric acid as eluent is shown in Fig. 1.

Application to digested samples

A chromatogram of a 125-fold dilution of an acetic acid–sulfuric acid-digested grain sample using the IC-Pak Cation column and nitric acid as eluent is shown in Fig. 2. The chromatogram shows a large negative void peak due to the low pH of the sample and 3.5 ppm of ammonium in the presence of traces of sodium and potassium. The grain samples typically contained 5–15% total nitrogen, hence the ammonium level in the digest was relatively high. Fig. 3 shows a chromatogram of a 1000-fold dilution of a potassium sulfate–sulfuric acid–digested organic compound (caprolactam) containing *ca.* 2% of total nitrogen obtained using the Protein-Pak SP-5PW column and 25 m*M* nitric acid–5% acetone as eluent. The potassium was typically present in a 50–100-fold excess over ammonium in such samples, hence the need for

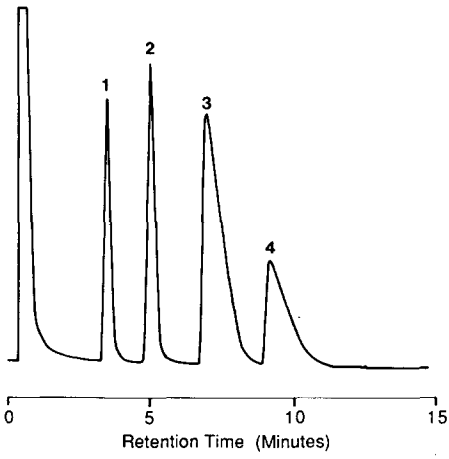

Fig. 1. Chromatogram of a monovalent cation standard using the IC-Pak Cation column. Eluent, 2 m*M* nitric acid; flow-rate: 1.2 ml/min; injection, 100 μl. Solutes: 1 = lithium (1.0 ppm); 2 = sodium (5.0 ppm); 3 = ammonium (10.0 ppm); 4 = potassium (10.0 ppm).

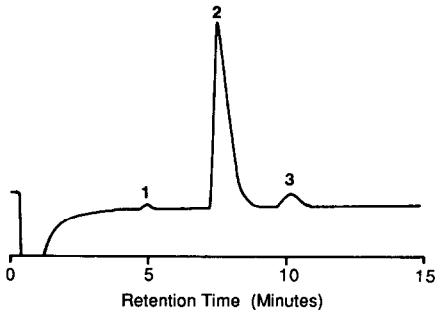

Fig. 2. Chromatogram of an acetic acid–sulfuric acid-digested grain sample using the IC-Pak Cation column. Conditions as in Fig. 1 except sample preparation: 125-fold dilution of acetic acid–sulfuric acid-digested grain. Solutes: 1 = sodium; 2 = ammonium (3.55 ppm); 3 = potassium.

the use of the higher ion-exchange capacity column. Blank digests were processed for both Kjeldahl digestion procedures and no detectable ammonium was found. No decrease in the performance of either column was observed as a result of injecting *ca.* 200 digested samples during the course of this work.

The results for total nitrogen obtained by the classical Kjeldahl and the digestion–IC methods were compared for ten acetic acid–sulfuric acid-digested grain samples. The results obtained by the two methods were normalized to account for differences in sample weights and are given in Table I. The two methods showed reasonably good agreement. A paired comparison Student's *t*-test [8] indicated no significant difference between the two sets of results at the 95% confidence limit. The average difference between the two sets of data pairs was 5.1%.

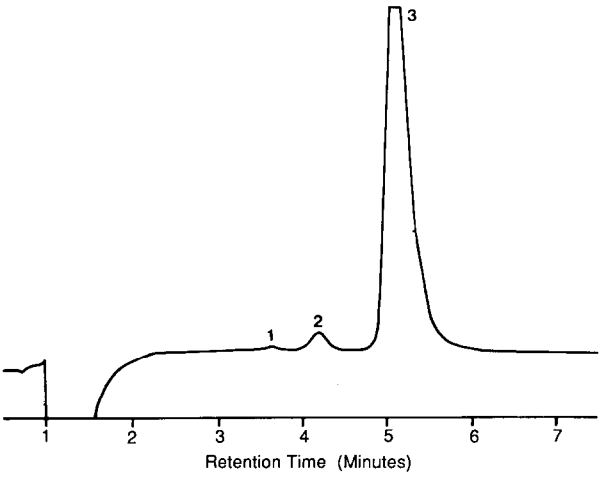

Fig. 3. Chromatogram of a potassium sulfate–sulfuric acid-digested sample of caprolactam using the Protein-Pak SP-5PW column. Eluent, 25 m*M* nitric acid–5% acetone; flow-rate, 2.4 ml/min; injection, 100 μl; sample preparation, 1000-fold dilution of potassium sulfate–sulfuric acid-digested caprolactam. Solutes: 1 = sodium, 2 = ammonium (0.45 ppm); 3 = potassium.

TABLE I

COMPARISON OF RESULTS FOR TOTAL NITROGEN OBTAINED BY THE CLASSICAL KJELDAHL METHOD AND BY THE DIGESTION–IC METHOD[a] FOR TEN ACETIC ACID–SULFURIC ACID-DIGESTED GRAIN SAMPLES

Sample No.	NH_4 in digest (classical method) (ppm)	Total N (classical method) (%)	NH_4 in digest[a] (IC method) (ppm)	Total N (IC method) (%)
1	302.7	12.33	338.1	13.77
2	141.9	5.76	137.2	5.57
3	322.5	13.0	310.9	12.53
4	305.9	12.15	297.6	11.82
5	140.7	5.76	136.6	5.50
6	182.2	7.40	199.8	8.12
7	306.7	12.33	281.7	11.32
8	319.2	13.00	306.8	12.50
9	303.8	12.15	292.8	11.71
10	182.5	7.40	175.3	7.10

[a] Average of triplicate determinations.

The results obtained by the classical Kjeldahl digestion–distillation–titration procedure for four caprolactam samples digested using the potassium sulfate–sulfuric acid method were compared with those obtained by the digestion–IC method. The average difference between the two set of results was 10.4%. The method was also applied to the determination of total nitrogen in sewage sludges digested using a potassium sulfate–sulfuric acid mixture with a mercury(II) oxide catalyst. The results of the digestion–IC method showed an average difference of *ca.* 14% compared with those obtained by the classical Kjeldahl digestion–distillation–titration procedure.

CONCLUSIONS

The use of ion chromatography to determine total nitrogen as ammonium ion after sample digestion improves the speed of the analysis compared with the conventional Kjeldahl method by eliminating the need for the distillation step. As only very small volumes of samples are required for analysis, the weight of sample required for the digestion can be reduced, minimizing the amount of catalyst required, hence also reducing disposal costs. The modified ion chromatographic–Kjeldahl method showed reasonable agreement with the results obtained by conventional methods for total nitrogen analysis; the variation was between 5 and 14% depending on the sample type and digestion procedure used. The method was applied to the determination of total nitrogen in foods, organic compounds and sewage sludges and should also be applicable to a wide variety of other sample types.

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