This paper presents a simple and rapid method for micro-Kjeldahl analysis. After sulfuric acid digestion, the ammonia is distilled into boric acid solution and titrated directly with 0.01 N hydrochloric acid, using methyl red-bromocresol green as indicator. Ten determinations can be completed in an hour. (The SCI® indicates that this paper has been cited over 310 times since 1961.)

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"Kjeldahl nitrogen determination is the most common method in the field of organic elemental analysis. It is carried out in a wide range of analytical laboratories such as agricultural, biochemical, clinical, and pharmaceutical laboratories. This method was discovered by Kjeldahl 90 years ago when he found that nitrogen in agricultural materials was converted into ammonium sulfate upon heating with concentrated sulfuric acid. He completed the analysis by liberating the ammonia with alkali, absorbing it in a known amount of standardized acid, followed by back-titration of the acid. In 1912, Pregl adapted this method to the micro scale to determine nitrogen in 3 to 5 milligrams of organic compounds, and the procedure became known as the micro-Kjeldahl method.

"Shortly before World War II, I took charge of the microchemical laboratory at the University of Chicago. Because there were only a few microanalysis laboratories in the US at the time, I received numerous requests from near and far for the analysis of a great variety of samples, among which were biochemical liquids and also solutions containing polymamines or fatty amines. The Kjeldahl method is suitable for analyzing these materials but the Pregl procedure could not be directly applied because the nitrogen compounds were in solutions of unknown concentrations within very wide ranges. It occurred to me that the micro-Kjeldahl method could be improved in several respects.

"Meanwhile, Guillermo Zuazaga came to Chicago from Puerto Rico. He wanted to learn microchemical techniques and re-search methods. Since he was to return to work in his native land, I suggested a research problem which did not require sophisticated and expensive equipment. First, Guillermo performed titrations of microamounts of ammonia with standardized acid solution hoping to find a suitable single indicator. When this was unsuccessful, I told him to try a combination of indicators. Employing a mixture of methyl red and bromocresol green, Guillermo was thrilled to discover that the titration end-point could be unequivocally located within 0.02 ml. (half a drop) of 0.01 N HCl. Then we undertook thorough investigation of the micro-Kjeldahl method and developed a simple, rapid, and precise procedure. By collecting the ammonia in boric acid solution instead of standardized acid, the need of standard alkali solution is eliminated. By using a compact steam-distillation apparatus with short connections, together with the direct titration technique, eight to ten determinations can be completed in an hour. I tested out the new procedure with my students and also asked other analysts to try it. One year later, I wrote up the method for publication, and it quickly received wide acceptance.

"I was pleasantly surprised that after more than 35 years, the Science Citation Index® identified this paper as one of the most cited items. My micro-Kjeldahl method has been described as the standard procedure in books and articles for a long time, frequently without giving the original literature source. I am grateful to the authors who have listed my paper for reference, and I do not blame those who have not. I may mention that this paper was my first publication on microchemistry, which is concerned with the principles and methods of chemical experimentation using the minimum quantity of working material to obtain the desired chemical information. Since then I have developed many microchemical procedures, always with a view to utilize simplified operations and inexpensive equipment to produce experimental results which are as precise and reliable as those obtained by expensive instruments and complicated processes."