

# Chromatography

## Traditional Amino Acid Analysis.

### What has Changed?

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During more than 10 years of focused research into traditional (or 'classical') amino acid analysis, scientists at JPP Chromatography Limited (a Plymouth, UK-based R&D Company) have made revolutionary discoveries that simplify and streamline the entire analytical process, whilst making no sacrifice to sensitivity, performance or speed. Newly formulated 'single bottle' ninhydrin reagents with long lives when stored in air at room temperature work extremely well on existing instruments – and even more effectively on instruments modified by making straightforward but clever design changes.



Novel chemical and physical processes outlined in 3 different patents that protect JPP's discoveries in key global territories allow ninhydrin reagents **excluding hydrindantin** in their preparation rapidly to produce the hydrindantin that is fundamentally needed for analysis to take place, when and where it is needed - in the heated reaction zone inside the instrument.

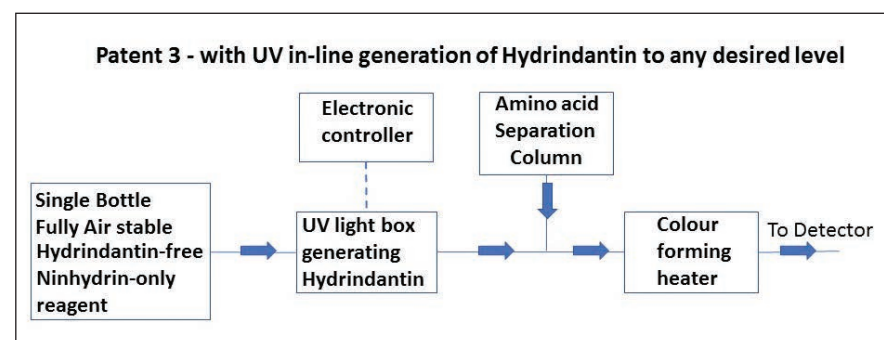
**Patent 1** protects a novel chemical process in the reagent only that allows its effective use on presently configured 'single heating chamber' instruments, whilst both **Patents 2 and 3** protect the use of even simpler hydrindantin-free reagents on instruments incorporating straightforward but totally new technical modifications that improve overall analytical performance and offer choice to the end user. **All JPP's reagents are insensitive to air and can safely be stored at room temperature for periods in excess of 3 years.**

A **Patent 1** reagent has been commercially produced by a leading industry participant since 2014 under non-exclusive licensing arrangements. It now has a clinically certified effective life of more than 3 years in air at room temperature. The reagent is in use in a number of leading medical and scientific establishments across 3 continents, including several UK NHS Hospitals.

**Patents 2 and 3** are now also available for license. These not only allow the use of long life, single bottle, hydrindantin-free reagents that are insensitive to air, but straightforward patented instrument modifications provide optimum sensitivity whilst delivering superior analytical performance.

**Patent 2** involves the use of a 'pre-heater' that most present commercial instruments will accommodate without significant modification. This allows acceleration of the production of hydrindantin within the instrument.

**Patent 3** uses a totally different analytical approach that avoids the need to heat the



reagent to a high temperature before sufficient quantities of hydrindantin are produced to allow analysis to take place. A simple reagent comprising just ninhydrin a solvent and a buffer is irradiated with UV light to generate chosen quantities of hydrindantin 'in-line' at room temperature. This new method is unique because it gives users total control of hydrindantin concentration immediately before exposure to the heated reaction coil. Concentrations of hydrindantin in-line are regulated electronically, either by analogue dial or digitally through computer software. Hydrindantin concentrations can instantly be changed to suit eluent flow rates and reaction times of the colour-forming coil. Detection sensitivities thus remain at maximum levels regardless of chromatographic conditions.

#### JPP offers:

- Simplicity – for producers and end users
- Time savings in the Lab
- Exceptional Air Stability of Reagents lasting 3 years+
- Consistent Sensitivity
- Unfailing Analytical Accuracy
- Versatility and Choice
- Profit Improvement

## AMINO ACID ANALYSIS ('AAA')

### A Brief History

In the late 1940s and early 1950s, two US scientists: Stanford Moore and William Stein (subsequent Nobel Prize winners for Chemistry) together with a colleague, Darryl Spackman, developed a 'simple' and rapid method for automatically recording the ninhydrin colour value of amino acids and amines in the effluent from ion exchange columns through the use of an instrument made for the purpose. More than 60 years later, their discovery: 'the Moore & Stein method' is still commonly regarded scientifically as the 'gold standard' for amino acid analysis.

## What is Amino Acid Analysis?

AAA is used around the world every day for the precise determination of protein quantities in tested samples. It is also used to provide detailed information on the relative amino acid composition and free amino acids in those samples. This is fundamental to the detection, isolation and analysis of numerous compounds of interest in many important fields including: clinical analysis, agriculture, food (human and animal), the environment, forensics, histochemistry and cell biology, pharmaceuticals, microbiology, medicine, nutrition, plant and protein sciences.

Amino acids are the building blocks of all living things. The ability to measure individual amino acids in samples is fundamentally important, not only for the health and well-being of human populations, but also in new areas of biological research. AAA is extensively used throughout the world in a clinical setting, being critical to the diagnosis of a range of rare metabolic disorders, particularly in new-born children. It is also used for a wider range of clinical diagnoses in people of all ages and is also increasingly used routinely and widely in the analysis of human and animal foods and liquids for quality control and monitoring freshness and spoilage.

## How does Analysis Take Place?

A sample submitted for analysis is introduced into an amino acid analyser - a purpose built precision liquid chromatography instrument - which separates it into individual amino acids before mixing with a 'reagent' (a pre-prepared liquid, always based on a compound called ninhydrin). The reagent and each separated amino acid are then heated in a chamber within the instrument to initiate a complex chemical reaction that creates an intensely coloured compound (Ruhemann's purple) which is detected and measured. The result is a computer-generated chromatogram, where each of up to 40 amino acids is identified and their concentration precisely calculated and recorded. The relative quantities of each recorded amino acid present in analysed samples allow interpretation of the data to be carried out by those with the requisite expertise in the relevant field.

## What are the Disadvantages of the Moore & Stein Method?

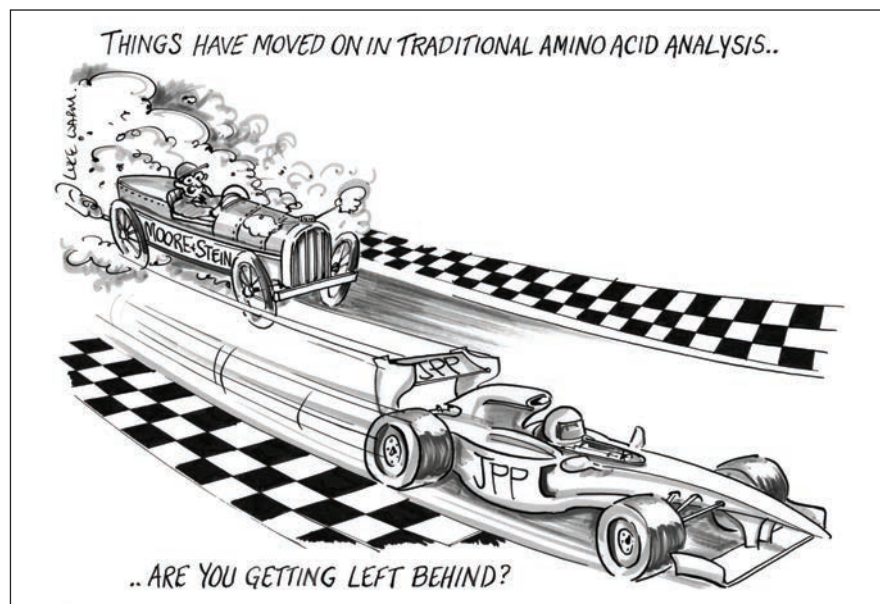
The ninhydrin reagent used in the Moore & Stein method always includes an inherently unstable component: hydrindantin, which enables analysis to take place. A two bottle 'kit' is therefore required, which virtually every major commercial manufacturer sells. The first bottle contains a concentrated solution of hydrindantin which must be mixed with the contents of the second bottle, which contains the more stable compounds or, alternatively, the second bottle is injected with a strong reducing agent using a syringe, in order to produce hydrindantin in situ immediately before analysis, so that an effective reagent can be created.

All this is time-consuming and laborious. Hydrindantin is fundamentally needed for rapid analysis to take place, but its intolerance to air is a major hindrance because it quickly degrades when put into use. Traditional AAA as it is carried out all over the world today, therefore, is costly and inconvenient for both manufacturers and end users. The very short lifetime of reagents after mixing leads to wastage, particularly for low volume users. But these handicaps have always been accepted, in the absence of other practical and effective ways to carry out analysis.

Despite its clear practical disadvantages, the Moore & Stein method is still adopted today by all commercial instrument and reagent manufacturers in the traditional AAA field. It has for more than 60 years provided excellent analytical performance, freedom from interference and exceptional robustness. It quickly became the 'gold standard' on its introduction in the early 1950s and it has remained so ever since.

## An Inflection Point has been Reached

JPP's discoveries remove the difficulties associated with Moore & Stein's method. They mark the passage to a new era for traditional methodology. The patents are available for license on negotiable terms, subject to contract. Ownership participation at corporate level would be considered, subject to applicant status.



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