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Product Information

Phthaldialdehyde Reagent
Complete Solution

Catalog Number **P0532**
Storage Temperature 2–8 °C

CAS RN 643-79-8 (phthaldialdehyde)
Synonyms: *o*-Phthaldialdehyde Reagent, OPA Reagent

Product Description

This product is specially formulated for precolumn derivatization of primary amines and amino acids prior to analysis by high pressure reverse-phase liquid chromatography.[1] The amino acid derivatives are usually detected by fluorescence (excitation wavelength = 340 nm and emission wavelength = 455 nm); however, less sensitive detection by absorbance at 340 nm can be performed.[2]

The reagent contains phthaldialdehyde (1 mg/ml), Brij® 35, methanol, 2-mercaptoethanol, potassium hydroxide, and boric acid, pH 10.4.

An example of an amino acid analysis, using freshly prepared reagent, is shown in Figure 1A. Experimental conditions are described in the figure legend.

Special care should be taken to ensure the product is maintained in an inert atmosphere (nitrogen or argon) at 2–8 °C since improper storage or handling conditions may render the solution ineffective. Figure 1B shows an example of an amino acid analysis using improperly stored reagent solution (the reagent was stored at 2–8 °C in the presence of oxygen for over ten months).

Phthaldialdehyde Solution, which has become oxidized, see Figure 1B, can easily be reduced by the addition of 0.5–5 µl of 2-mercaptoethanol (Catalog Number M6250) per ml of undiluted Phthaldialdehyde Reagent. Addition of more than 5 µl per ml decreases the sensitivity of the reagent. An example of an amino acid analysis using the same reagent employed in Figure 1B, after reduction with 2-mercaptoethanol, is shown in Figure 1C.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product in an inert atmosphere (nitrogen or argon) at 2–8 °C.

References

1. Jones, B.N., et al., J. Liq. Chromatog., **4**, 565 (1981).
2. Svedas, V.K., Anal. Biochem., **101**, 188 (1988).

Figure 1.
Elution Profile of Amino Acid Standards Derivatized with Phthaldialdehyde Reagent

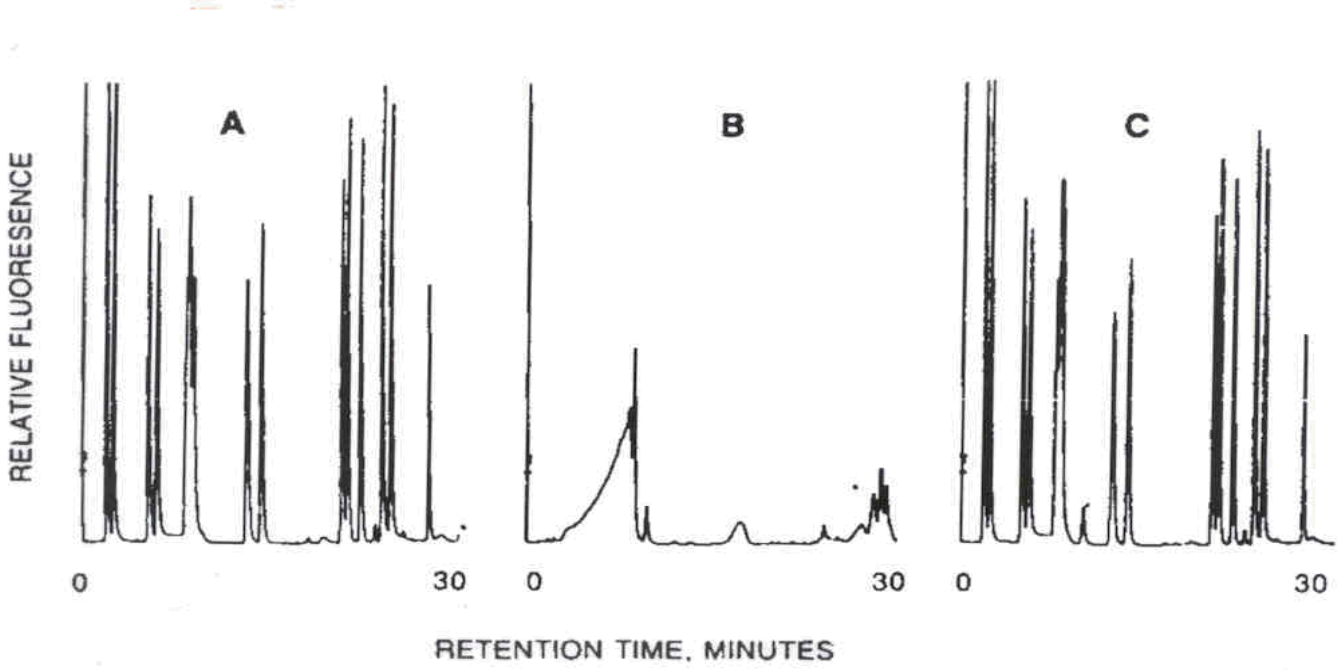

Figure 1A.
Reaction of amino acid standards with freshly prepared Phthaldialdehyde Reagent.

Figure 1B.
Reaction of amino acid standards with ten month old Phthaldialdehyde Reagent that had been improperly stored.

Figure 1C.
Reaction of amino acid standards with the same Phthaldialdehyde Reagent employed in Figure 1B after the addition of 2 µl of 2-mercaptoethanol per ml of Phthaldialdehyde Reagent.

Reaction conditions - An equal volume of Phthaldialdehyde Reagent and amino acid standard solution (Catalog Number AAS18, diluted 100-fold with 0.001 N HCl) were mixed together at room temperature.

Chromatographic conditions - After 1 minute, 0.01 ml of the reaction mixture was injected onto a 4.6 mm × 15 cm C18 reverse-phase column. After injection, the gradient program was:

40% B for 0.5 minute
17 minute concave gradient to 50% B
15 minute linear gradient to 100% B
followed by a 5 minute isocratic elution with 100% B

Solvent A - methanol-tetrahydrofuran-50 mM phosphoric acid, (20:20:960) pH adjusted to 7.5 with NaOH
Solvent B - methanol-water, (65:35).

The flow rate was 1.5 ml/minute at ambient temperature. Amino acid derivatives, at 125 pmole each, were detected by fluorescence.

Under these conditions, cysteine and ammonia give very low fluorescence and proline is undetectable.[1]