INT-PYD
Acetylated Pyridinoline
HPLC Internal Control

QUIDEL

For the determination of the recoveries of bone collagen crosslinks, pyridinoline (PYD) and deoxypyridinoline (DPD) in the pretreatment of the samples prior to analysis by HPLC.

For **Research Use Only**. Analytical and performance characteristics have not been established.

Read the entire Package Insert thoroughly before using this reagent. The Int-Pyd should be stored at ≤–20°C until use.

INTENDED USE

The Pyridinoline Internal Control(Int-Pyd) is designed for the determination of the recoveries of bone collagen crosslinks, pyridinoline (PYD) and deoxypyridinoline (DPD) in the pretreatment of the samples prior to analysis by HPLC. The recoveries of PYD and DPD after pretreatment vary greatly depending on the nature of the sample and the variability of the pretreatment conditions used by different laboratories. The determination of absolute concentrations of PYD and DPD in biological fluids requires an assessment of their recoveries in the pretreatment steps of the HPLC analysis.

SUMMARY AND EXPLANATION

Int-Pyd is a chemically modified pyridinoline (acetylated pyridinoline) that has the same physico-chemical properties in the sample pretreatment as PYD and DPD and therefore adequately represents recoveries of PYD and DPD. The retention time of Int-Pyd in HPLC is typically greater than that for PYD and DPD. This allows for good HPLC peak separation and is convenient for the monitoring of recoveries. Recoveries of the Int-Pyd spiked into the individual samples accurately represents recoveries of PYD and DPD in normal and diseased urine samples with various creatinine levels.

MATERIALS PROVIDED

Cat. #8006

Component	Part No.	Qty/Volume
Int-Pyd	4102	1 x 5 mL

Int-Pyd is provided as a “20x” solution in 90% Acetic Acid.

WARNINGS AND PRECAUTIONS

- Research Use Only. Analytical and performance characteristics are not established.
- Pyridinoline is purified from human or bovine material and should be treated as potentially biohazardous.
- Acetic acid is poisonous, corrosive and can cause severe burns. Avoid contact with skin, eyes, and clothing. May be fatal of swallowed. Harmful if inhaled. Combustible. Keep away from heat and flame.
- Int-Pyd should be disposed of in a manner consistent with relevant regulations.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND STABILITY

- Store Int-Pyd solution at ≤ –20°C when not in use.
- Pyridinoline is photosensitive; avoid prolonged exposure to light.
- Int-Pyd is stable for up to 7 days at room temperature, if not exposed to light. Provided light exposure is kept at a minimum, product may undergo up to 5 freeze/thaw cycles.
- Int-Pyd is susceptible to hydrolysis at low pH and should not be exposed to strong acids (e.g. 6N HCl) for prolonged periods of time.

PROTOCOL

1. Prepare 100-200 µL of a 1x solution of 20x Int-Pyd in 2% Heptafluorobutyric Acid (HFBA). (DO NOT inject 20x solution of Int-Pyd directly on HPLC.)
2. Inject 50-100 µL of 1x solution onto RP-C_{18} HPLC column equilibrated with the mobile phase used in Pyd and Dpd analysis. Develop column with gradient of CH_3CN as in HPLC analysis for Pyd and Dpd and monitor fluorescence (ex 295nm, em 395nm). For absolute calibration of fluorescent signal, use Pyd/Dpd HPLC Calibrator, Quidel Catalog Number 8004.
3. Calculate the integrated fluorescence of Int-Pyd per 1 µL of 1x solution of Int-Pyd.
4. Determine the appropriate amount of Int-Pyd spike required for the analysis of Pyd and Dpd according to your sample pretreatment protocol.

REFERENCE PRETREATMENT PROCEDURE

1. To 0.6 mL of urine sample hydrolyzed overnight in 6 NHCl, add 2.4 mL of n-butanol and 0.6 mL of 1x solution of Int-Pyd in 90% acetic acid (NOTE: Do not dilute Int-Pyd with 100% acetic acid).
2. Apply mixture onto the cellulose cartridge (100 µg dry weight) and wash cartridge with butanol:acetic acid: water (v:v:v:) mixture.
3. Elute Pyd and Dpd with water and use a portion of the eluted material for RP-HPLC.
4. Determine total relative fluorescence of the Int-Pyd spiked into the sample (steps 1-4 of protocol above) and the total relative fluorescence of the Int-Pyd eluted from cellulose cartridge and calculate the Int-Pyd recovery.
5. Use the obtained recovery to calculate Pyd and Dpd concentrations in the urine sample.

REFERENCES

1. Black, D., A. Duncan, and S.P. Robins. 1988. Quantitative Analysis of the Pyridinium Crosslinks of Collagen in Urine Using Ion-Paired Reverse-Phase High-Performance Liquid Chromatography. *Analytic Biochem*. 169:197-203.
2. Pratt, D., Y. Daniloff, A. Duncan, and S.P. Robins. 1992. Automated Analysis of the Pyridinium Crosslinks of Collagen in Tissue and Urine Using Solid-Phase Extraction and Reversed-Phase High-Performance Liquid Chromatography. *Analytic Biochem*. 207:168-175.

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