Waters Lab Highlights

An Internal Communication of Applications and Techniques

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The Identification of "Mystery Peaks" in Physiological Pico•Tag Analysis

The identification of all peaks resulting from a Pico•Tag® amino acid analysis of a physiological sample such as plasma or urine can be complicated by the presence in these samples of unusual amino acids or non-amino acid compounds which derivatize and/or have absorbance near 254nm. Another aspect of this problem is the need to identify substances which are suspected to be present in the samples, such as drugs and their metabolites and unusual dietary components.

In order to simplify this task we included in the Pico•Tag Method (Literature #WM-02) several lists of compounds which have been identified along with their elution positions in the Pico•Tag separation. Portions of selected tables are reproduced here for use in the examples.

How to use the tables:

I. To identify a peak which does not correspond to a known amino acid in the standard mix:

- Take the ratio of the unknown peak's retention time to Alanine (Ala) or to one of the internal standard peaks, norleucine (Nle) or methionine sulfone (MetSO). This is the relative R.T. value.
- Using the list of compounds in order of retention (Table 2-7) locate the compound whose relative retention time is closest to that of the unknown.

Example: If the unknown retention time is 52.83 min. and Ala retention is 21.52 min. then the relative retention is 2.455 and the unknown peak may be heparin (Figure 1). Verify the ID by making sure that surrounding peaks clearly

Figure 1: Table 2-7. List of Compounds in Order of Retention

(Calibration Standard Components are highlighted to facilitate locating unknowns)

Compound	Rel. to Nle	Rel. to MetSO	Rel. to Ala
Valine	**0.821**	**1.896**	**2.310**
Diaminopimelic Acid I	0.847	1.956	2.383
Methionine	**0.850**	**1.962**	**2.390**
Diaminopimelic Acid II	0.856	1.976	2.407
Heparin	*0.873*	*2.016*	*2.455*
3-Hydroxy-Kynurenine	0.879	2.030	2.472
Lanthionine	0.886	2.047	2.493
Cystathionine	**0.892**	**2.060**	**2.509**
Phenobarbitol	0.909	2.100	2.557

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correspond to known peaks in the standard mix. It is also a good idea to double check the ID by calculating the relative retention time to both Ala and an internal standard peak.

II. To locate a compound which you believe to be in your sample (such as a medication, or an unusual amino acid not found in the calibration standard):

- Using the alphabetical list of compounds (Table 2-8) locate the component of interest and note its relative R.T. to alanine and/or the internal standard used in the separation.

- Return to the list of compounds in order of retention and locate the entry. Note which standard components it elutes nearest and then search your sample chromatogram for unidentified peaks in that area. Verify the ID by calculating relative R.T. for the unknown peak and compare the value to that in the table.

If you do not find your component of interest in the alphabetic list try the lists of compounds which are either eluted very early or are eluted in the wash after Lysine. (Tables 2-15 and 2-16).

Example: A plasma or urine sample is suspected to contain procainamide (a drug used to treat arrythmias). The relative R.T. for procanamide to Ala is 3.120 (Figure 2) which places it between the Orn and Lys peaks in the standard. Since there are several other possible ID's for a peak in that region be careful to verify the relative R.T. and try to rule out the presence of other substances which elute in that area.

Figure 2: Table 2-8. List of Compounds in Alphabetical Order

Compound	Rel. to Nle	Rel. to MetSO	Rel. to Ala
Phosphothreonine	0.083	0.192	0.233
Phosphotyrosine	0.104	0.239	0.292
Procainamide	*1.109*	*2.561*	*3.120*
Proline	0.475	1.098	1.337

There are occasionally compounds which coelute either with amino acids which are normally present in samples or with other unknowns. Therefore, it is not wise to rely solely on retention time data to make identifications. Other factors to consider are the amount of the component expected, the stability of the compound itself and of its PTC derivative and any unusual treatment of the sample.

Disclaimer: Not all unknowns are listed, some compounds not listed may co-elute with ones that are. Verify ID's by spiking suspected compounds into the standard mix and/or the sample. All data tabulated in The Pico•Tag Method comes from runs using the standard Pico•Tag gradient and eluents. Other conditions will give different values for relative retention time (R.T.). Drug metabolites may have different retention times than the unmetabolized drug and may appear as multiple peaks.

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