How to verify a pI calculation

The pI for a simple compound can be calculated by averaging the two pKa's that are relevant to the concentration of the molecule in its neutral charge state. For example, for glutamate:

$$pI = \frac{pK_1 + pK_R}{2} = \frac{2.19 + 4.25}{2} = 3.22$$

Since the pI is the pH where the average charge of the molecule in solution is zero, we can verify our calculated pI by calculating the average charge of glutamate in solution at pH 3.22. It should equal zero.

How do we calculate the average charge on glutamate?
We can calculate the average charge on each ionizable (acidic) group of glutamate, then sum these charges.

How do we calculate the average charge on one ionizable group?
We use the Henderson-Hasselbalch equation to determine the ratio of the charged to uncharged group in solution, then we determine the fraction charged. Multiplying the fraction charged by the value of the charge gives the average charge.

Let's do it!
Glutamate has 3 ionizable groups:

- α-carboxyl (α-COOH, $pK_1 = 2.19$)
- α-amino (α-NH_3^+, $pK_2 = 9.67$)
- R-group carboxyl (R-COOH, $pK_R = 4.25$).

<u>α-COOH:</u>

ratio: $\frac{[COO^-]}{[COOH]} = 10^{pH-pKa} = 10^{3.22-2.19} = 10^{1.03} = 10.7 = \frac{10.7}{1}$

fraction charged: $\frac{[COO^-]}{[COOH]+[COO^-]} = \frac{10.7}{1+10.7} = \frac{10.7}{11.7} = 0.915$

average charge: $0.915 \times -1 = \mathbf{-0.915}$

<u>α-NH_3^+:</u>

ratio: $\frac{[NH_2]}{[NH_3^+]} = 10^{pH-pKa} = 10^{3.22-9.67} = 10^{-6.45} = 3.55 \times 10^{-7} = \frac{3.55\times10^{-7}}{1}$

fraction charged: $\frac{[NH_3^+]}{[NH_3^+]+[NH_2]} = \frac{1}{1+3.55\times10^{-7}} = 1.00$

average charge: $1.00 \times +1 = \mathbf{+1.00}$

<u>R-COOH:</u>

ratio: $\frac{[COO^-]}{[COOH]} = 10^{pH-pKa} = 10^{3.22-4.25} = 10^{-1.03} = 0.0933 = \frac{0.0933}{1}$

fraction charged: $\frac{[COO^-]}{[COOH]+[COO^-]} = \frac{0.0933}{1+0.0933} = \frac{0.0933}{1.0933} = 0.0853$

average charge: $0.0853 \times -1 = \mathbf{-0.0853}$

Now we sum the average charges of each ionizable group in solution at pH 3.22 to find the average charge of glutamate at pH 3.22:

$$(-0.915) + (+1.00) + (-0.0853) = \mathbf{0.00}$$

Since the charges sum to zero, 3.22 is the correct pI!