

Table 1. The symbols and levels of the CCD test design for chlorpyrifos extraction from the urine by liquid-liquid microextraction method

| Factors | $\alpha+$ | 1+ | Zero | 1- | $\alpha-$ |
|--|-----------------------------|-----------|-------------|-----------|-----------------------------|
| Methanol (μL) | 1000 | 800 | 600 | 400 | 200 |
| Salt (%) | 4 | 3 | 2 | 1 | 0 |
| Surfactant (%) | 4 | 3 | 2 | 1 | 0 |
| Toluene (μL) | 525 | 450 | 375 | 300 | 225 |

Table 2. Optimization results of CCD-dispersive liquid microextraction method to increase the outcome of chlorpyrifos extraction from urine

| Test No. | Volume of dispersive solvent (μL) | Extraction solvent volume (μL) | Surfactant Concentration (%) | Salt concentration (%) | Chlorpyrifos outcome |
|----------|--|---|------------------------------|------------------------|----------------------|
| 1 | 800 | 400 | 3 | 3 | 90.63 |
| 2 | 600 | 300 | 4 | 2 | 75.91 |
| 3 | 600 | 100 | 2 | 2 | 63.09 |
| 4 | 1000 | 300 | 2 | 2 | 81.05 |
| 5 | 400 | 400 | 1 | 3 | 56.81 |
| 6 | 600 | 300 | 2 | 2 | 95.64 |
| 7 | 800 | 200 | 3 | 1 | 70.11 |
| 8 | 800 | 400 | 1 | 3 | 73.30 |
| 9 | 600 | 300 | 2 | 2 | 88.12 |
| 10 | 600 | 300 | 2 | 2 | 89.94 |
| 11 | 600 | 300 | 2 | 2 | 86.18 |
| 12 | 400 | 400 | 3 | 1 | 42.40 |
| 13 | 400 | 200 | 1 | 1 | 45.48 |
| 14 | 400 | 400 | 1 | 1 | 42.52 |
| 15 | 800 | 400 | 1 | 1 | 80.94 |
| 16 | 400 | 200 | 3 | 1 | 51.81 |
| 17 | 600 | 300 | 2 | 0 | 31.23 |
| 18 | 200 | 300 | 2 | 2 | 36.99 |
| 19 | 400 | 400 | 3 | 3 | 62.24 |
| 20 | 600 | 300 | 2 | 4 | 66.00 |
| 21 | 800 | 400 | 3 | 1 | 81.16 |
| 22 | 600 | 300 | 2 | 2 | 84.70 |
| 23 | 600 | 300 | 0 | 2 | 64.01 |
| 24 | 800 | 200 | 1 | 3 | 61.90 |
| 25 | 400 | 200 | 1 | 3 | 60.30 |
| 26 | 800 | 200 | 3 | 3 | 75.81 |
| 27 | 600 | 500 | 2 | 2 | 70.70 |
| 28 | 800 | 200 | 1 | 1 | 59.62 |
| 29 | 600 | 300 | 2 | 2 | 90.08 |
| 30 | 400 | 200 | 3 | 3 | 61.90 |

Table 3. Primary validation factors of the liquid-liquid microextraction method

| Indices | Values |
|---|---------------|
| R2 coefficient of determination | 0.956 |
| Adjusted coefficient of determination | 0.934 |
| Standard deviation of experimental error | 3.91 |
| The model coefficient of variation | 6.56 |
| Mean | 59.70 |
| Pred R-Squared | 0.872 |
| Adeq Precision | 19.086 |
| PRESS | 859.535 |

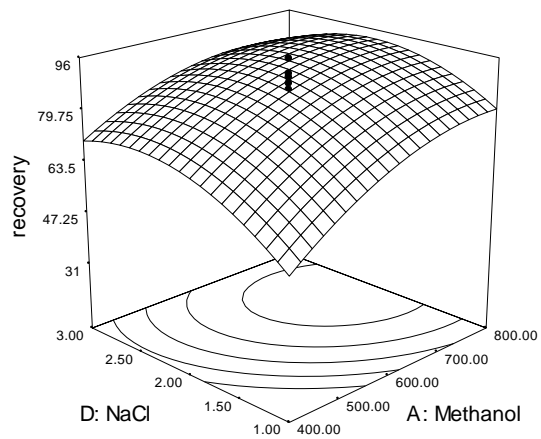
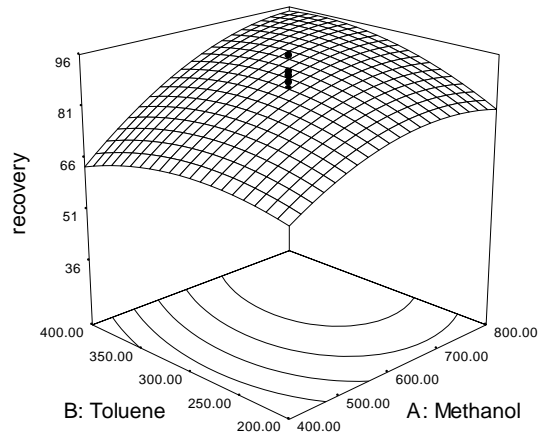


Figure 1. The results of response surface plots of the factors effective in increasing the chlorpyrifos extraction outcome from urine samples

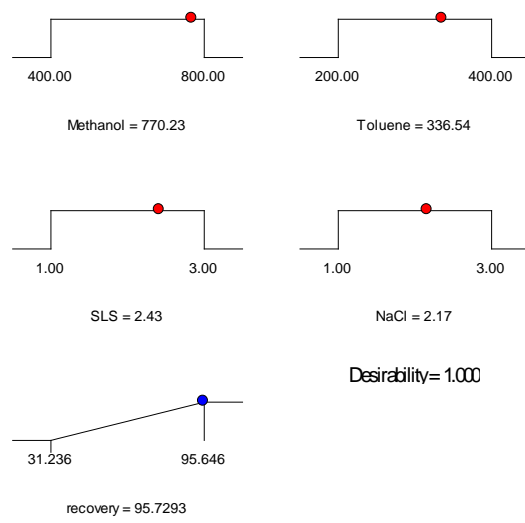


Figure 2. Schematic representation of the optimal values of the factors, responses, and associated levels

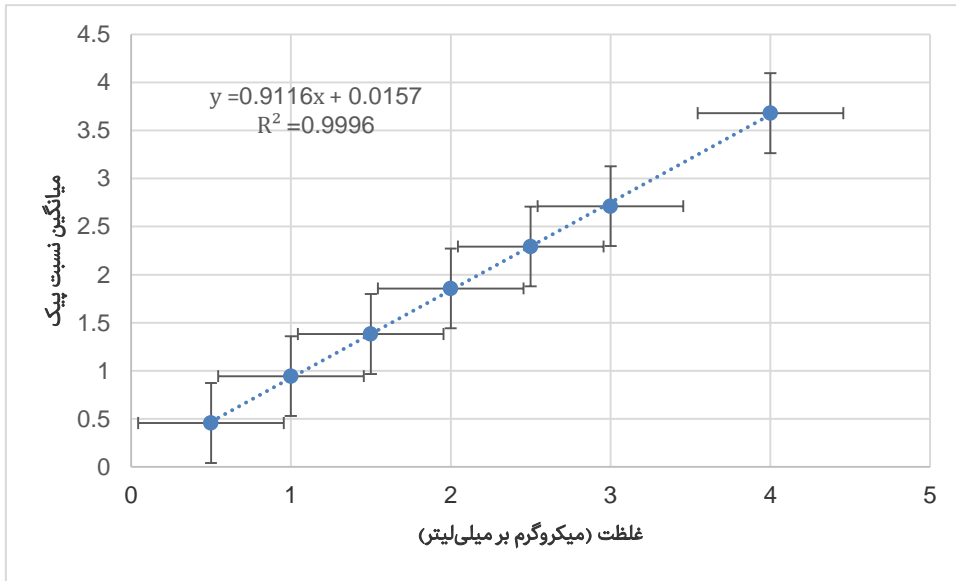


Figure 3. The chlorpyrifos calibration curve for urine sample

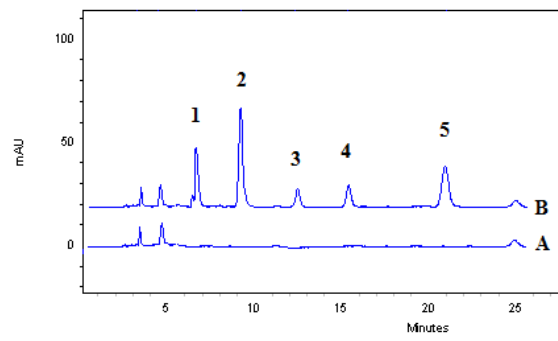


Figure 4. Chromatogram of the selectivity of chlorpyrifos in urine sample
A: Urine chromatogram without sample; B: Chromatogram with injected sample 1-Tramadol, 2-azinephosphate, 3-diazinone, 4-pyrimiphosmethyl, 5-chlorpyrifos