Application Note

NNAL analysis in human urine using AFFINIMIP®SPE NNAL

PROTOCOLE OF PURIFICATION

Sample preparation

100 mL of urine centrifuged at 4000 RPM during 10 minutes. Supernatant is then put to pH 6-7 with acetic acid and spiked (4 µg/L).

Purification with a 3mL AFFINIMIP® SPE NNAL cartridge

EQUILIBRATION

- 1mL DCM
- 1mL methanol
- 1mL ultrapure water

LOADING

- 5 mL of spiked urine

WASHING OF INTERFERENCES

- 2 mL ultrapure water

Drying 10 min under full vacuum

Washing of interferences

- 1mL Toluene
- 1 mL Toluene/DCM (9/1, v/v)
- 1 mL Toluene/DCM (4/1, v/v)

Drying 2 min under full vacuum

Elution

- 2 Methanol/DCM (1/9, v/v)

Elutions are then evaporated under vacuum during 10-15 min, and dissolved in 1 mL of mobile phase.

HPLC Metod with LC - MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)

- **Column**: Hypersil Gold 150*2.1mm ID (3µm) + guard column 10mm
- **Column temperature**: 30˚C
- **Flow rate:** 0,3mL/min
- **Injection volume:** 20µL

Mass parameters:

- Ion source: ESI Positive
- Curtain gas: 20
- Collision gas: Medium
- IonSpray voltage: 5500 V
- Source temperature: 500˚C
- GS1: 50
- GS2: 50

Table 1. HPLC gradient for the analysis.

Time (min)	Ammonium Acetate 10mM pH=6,1	Acetonitrile
0	95	5
1	95	5
5	71	29
7	30	70
8	30	70
9	95	5
13	95	5

Table 2. MRM transitions for the analysis

Analyte	Q1	Q3	DP (V)	EP (V)	CE (V)	CXP (V)
NNAL Q	210.2	93.1	86	10	31	6
NNAL q	210.2	180.1	86	10	17	14

RESULTS

Figure 1. Typical chromatogram obtained after clean-up.

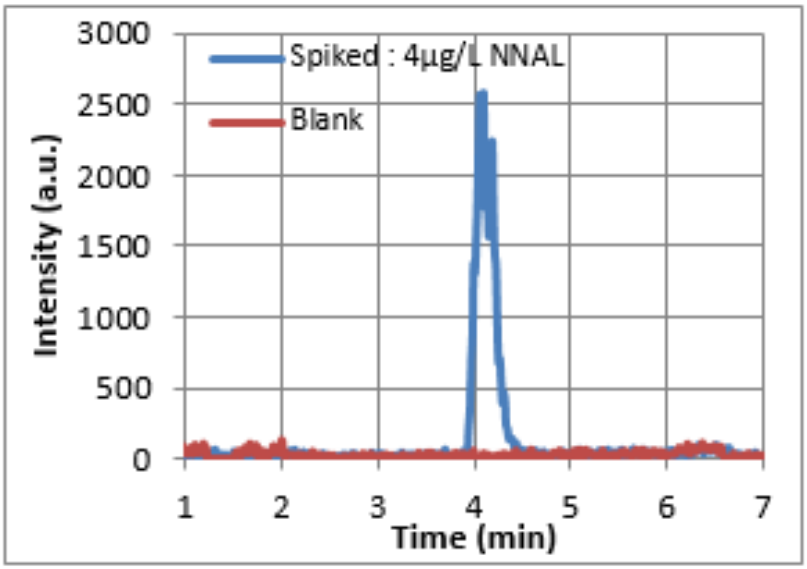

Table 3. Recovery at a concentration of 4 µg/L

Analyte	Recovery (%)	RSDr (%) (n=5)
NNAL	97	7

Good Recovery (> 90%)

Good repeatability (RSDr < 10%)

Product references :

AFFINIMIP®SPE NNAL cartridges :

- **3mL format**
  DG103-03 for 50 cartridges
- **10mL LRC**
  DG103-03LRC for 50 cartridges

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