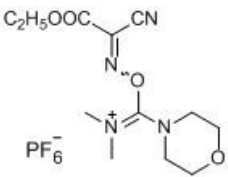


Qualitative HPLC Method for the Determination of COMU***Description of material***

Commercial Name:	COMU
IUPAC Name:	Morpholinium, 4-[[[(1-cyano-2-ethoxy-2-oxoethylidene)amino]oxayl] (dimethylamino)methylene]-hexafluorophosphate (1-) (1:1)
CAS RN:	1075198-30-9
Formula:	C ₁₂ H ₁₉ F ₆ N ₄ O ₄ P
MW	428,3 g/mol
Structure:	 <p>C₂H₅OOC-C(=O)-N=C(N)O-</p>

Outline of the method

The analysis of COMU purity is carried out by HPLC by means of a Zorbax RX-C8 column (or equivalent) through area normalization.

Scope of the method

HPLC determination of COMU quality.

Reagents

- Water (HPLC Grade)
- Acetonitrile (HPLC Grade)
- Trifluoroacetic acid (TFA, HPLC Grade)
- COMU: reference material or Standard
- Oxyma (ethyl (2E)-cyano(hydroxyimino)ethanoate): reference material or Standard

Instrument

HPLC with auto-injector and a PDA or UV detector capable to measure at 225 nm.

Operating conditions

Column: ZORBAX RX-C8 250x4.6mm 5µm PN 880967-901 Agilent

Regime:

Gradient

Pump program:

Time (min)	Water + 0.1% TFA	Acetonitrile + 0.1% TFA
0.00	75.0	25.0
6.00	75.0	25.0
11.00	15.0	85.0
15.00	75.0	25.0
20.00	75.0	25.0

Sample diluent:

Acetonitrile

Flow rate:

2.0 mL/min.

Column temperature:

Room Temperature

Detector wavelength:

225 nm

Injection volume:

10 µL

Retention Time:

~ 3.0 min.

Run time:

20.0 min

Method

1. Method

Preparation of mobile phase solutions:

Mobile phase A: Carefully add 1 mL TFA into 1 Liter of water and mix well (0.1 % (v/v) TFA in water).

Mobile phase B: Carefully add 1 mL TFA into 1 Liter of acetonitrile and mix well (0.1 % (v/v) TFA in acetonitrile).

2. Sample preparation

Reference or standard material and analyte sample preparation:

Weigh 8 mg of COMU reference or standard material and 2 mg *Oxyima* in a vial (20 mL), add 20 mL of acetonitrile. Place in an ultrasonic bath for one minute to dissolve. Transfer to an autosampler vial (2.0 mL) and inject by using the above described gradient program.

Sample preparation:

Weigh 8 mg of sample in a vial (20 mL), add 20 mL of acetonitrile. Place in an ultrasonic bath for one minute to dissolve. Transfer to an autosampler vial (2.0 mL) and inject by using the above described gradient program.

System equilibration:

Pump the mobile phase through the column under initial operating conditions until a stable baseline is obtained, then inject acetonitrile blank samples repeatedly until a chromatogram similar to that in shown in Figure 1 is obtained.

Calculations:

Calculate the percent of normalized area for the peak corresponding to COMU only.

Safety measures

- Read and understand the Material Safety Data Sheets for all compounds and reagents utilized. In case of doubt consult with your supervisor.
- Wear laboratory approved gloves, safety glasses and suitable protective clothing when handling any of these materials and reagents.
- Weigh and prepare the solutions under an efficient chemical hood.

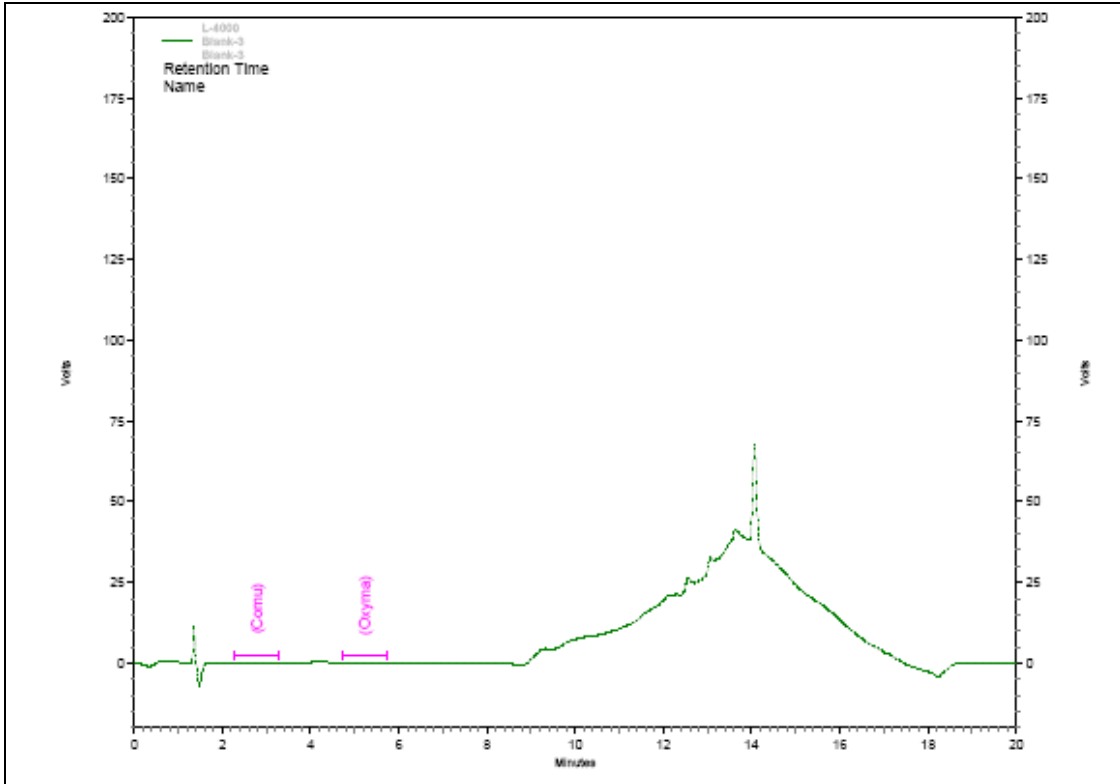


Figure 1. Typical chromatogram for the blank (acetonitrile).

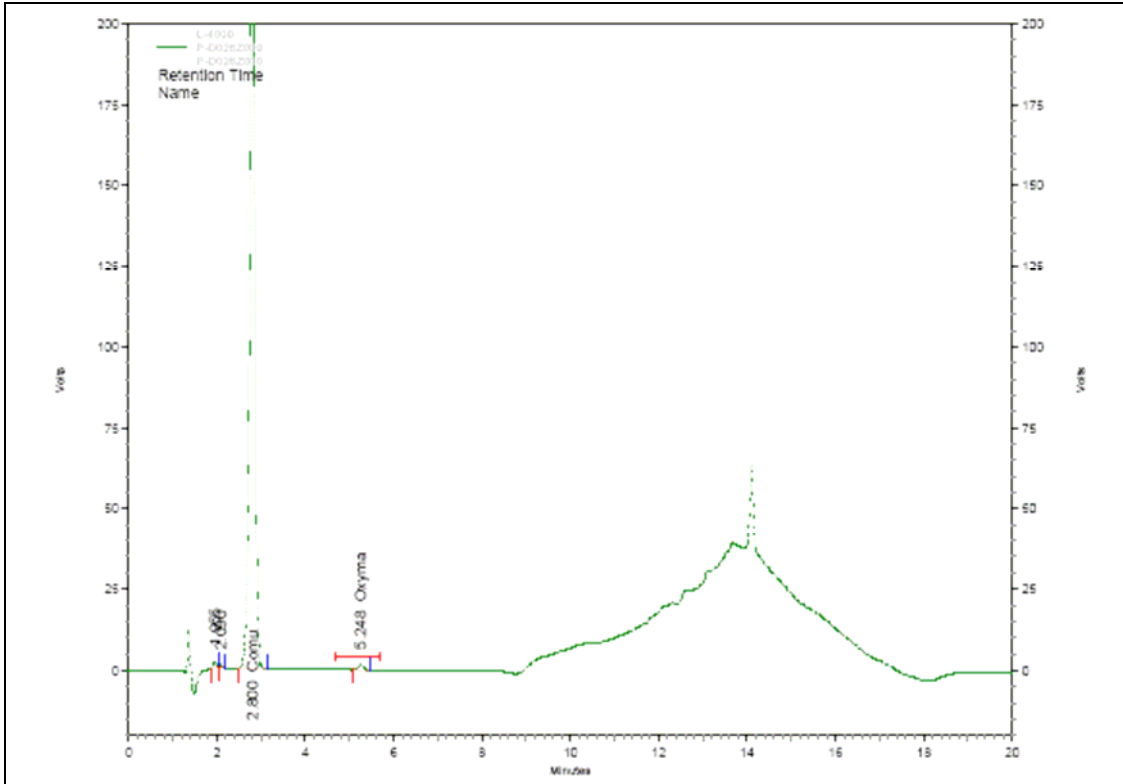


Figure 2. Typical chromatogram for a COMU sample.

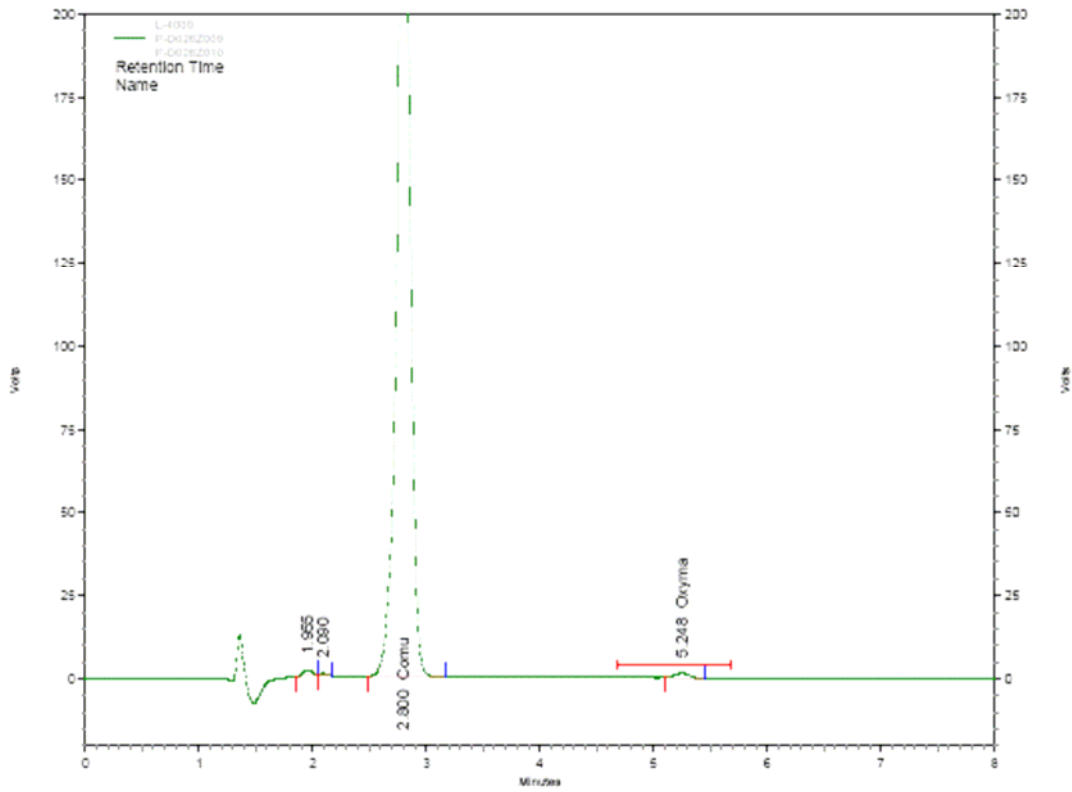


Figure 3. Typical chromatogram for a COMU sample (displaying only 8 minutes of the run).

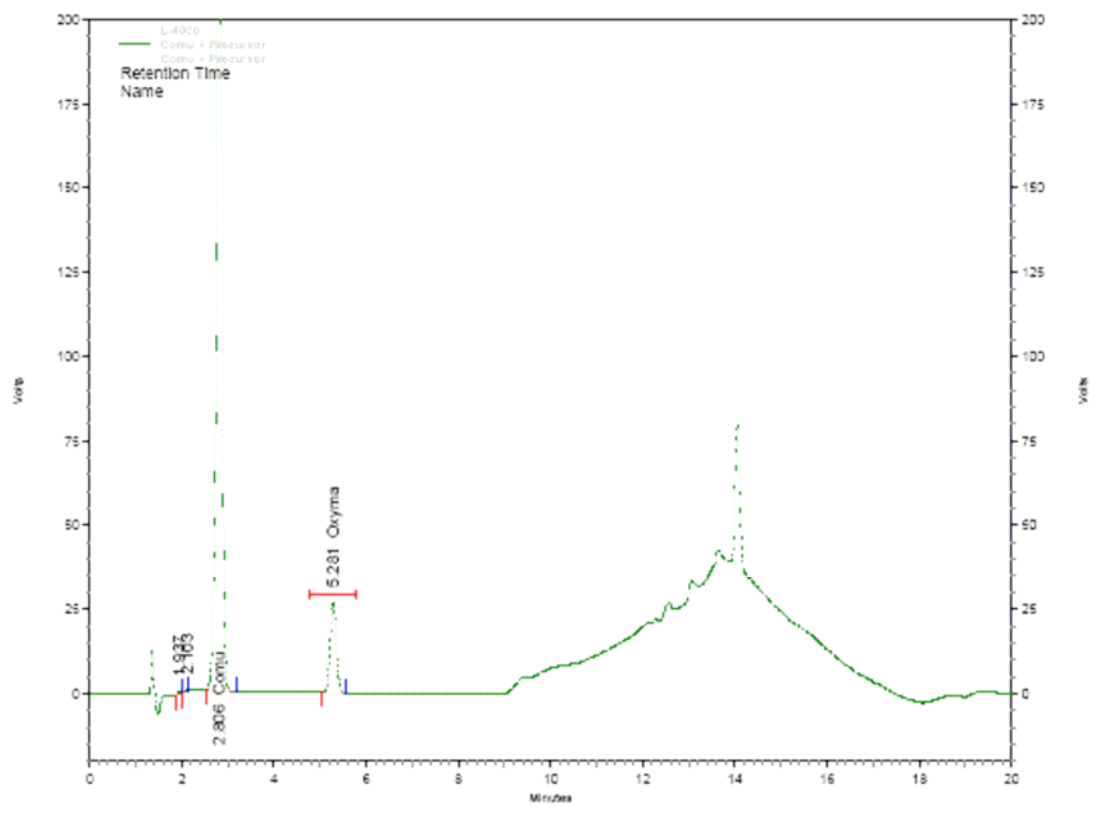


Figure 4. Typical chromatogram for a COMU sample + Oxyma spike.