Antec Scientific

Application Note
Drugs & Pharmaceuticals

Clozapine

The most reliable LC-EC applications for Drugs & Pharmaceuticals analysis

Antipsychotic drugs
Clozapine
Olanzapine
Risperidone

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Pharmaceuticals, API
Acetaminophen
Artemether
Artemisinin, Dihydro-artemisinin
Betadex sulfobutyl ether sodium
Etoposide
Epinephrine
Heparin
mesna BNP7787
8-OH-DPAT
Vincristine
Sulfides
Glutathione
Aminothiols
Disulfides

Aminoglycoside drugs
Amikacin
Framycetin sulphate
Gentamicin sulphate
Kanamycin
Netilmycin
Neomycin sulfate
Spectinomycin
Lincomycin
Tobramycin

- **Electrochemical detection of antipsychotic drugs**
- **Wall-jet flow cell with HyREF™ (Pd/H_2) electrode**
- **Reproducible & sensitive**

Introduction

Clozapine is a benzodiazepine and belongs to the class of atypical antipsychotic drugs used in the treatment of schizophrenia. Due to potentially fatal side-effects of high plasma drug levels, the United States Food and Drug Administration require monitoring of white blood cell count in patients receiving this drug. Clozapine is usually used as a last resort in patients that have not responded to other anti-psychotic treatments due to the side-effect and costs associated with the requirement of blood tests continually during treatment.

Plasma levels of Clozapine can be quantified using an electrochemical detector after sample pretreatment and HPLC separation [1]. In this application note a method (proof of principle) is presented for the analysis of Clozapine standards which demonstrates the applicability of the ALEXYS LC-ECD system with DECADE II electrochemical detector for the analysis of Clozapine.

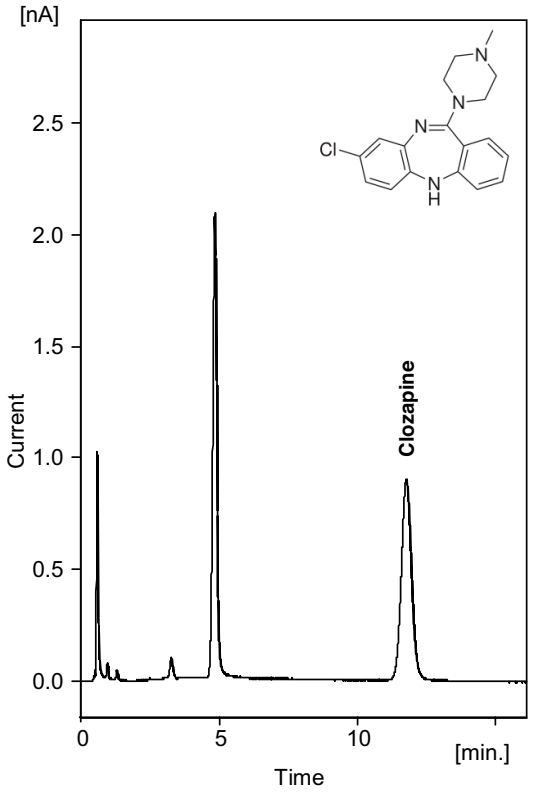

Figure 1: Analysis of a 50 ng/mL Clozapine standard in mobile phase. Measurement conditions as given in Table 1. The peak at 5 min is from 50 ng/mL Olanzapine which was also mixed in this standard.

Figure 2: ALEXYS analyzer

Method

For the separation of Clozapine, the use of C18 columns with a mobile phase containing 60-82% organic modifier has been reported in literature [1, 2]. For electrochemical detection after LC separation it is recommended to have at least 10 mM ions in the mobile phase [3]. Clozapine standards in the range of 0.3-100 ng/mL were prepared in mobile phase and used to assess optimal working potential, linearity, repeatability, and detection limit.

Conditions

Table 1 gives the conditions that were used to measure the reported results unless stated otherwise. As this application note is a proof of principle for the analysis of Clozapine standards with ECD, it is not particularly a set of conditions optimized for the analysis of plasma samples. A full method for Clozapine and its active metabolites including sample pretreatment is described in reference [1].

Table 1

Conditions	
Mobile phase	Phosphate buffer 50 mM set to pH 6.5, 25% methanol, 25% acetonitrile
Column	C18, 50 x 1 mm ID, 3 µm particle size
Flow rate	50 µL/min
Injection volume	1 µL
Needle wash	100% acetonitrile
Temperature	35 °C
Flow cell	SenCell 2 mm GC HyREF, spacing position 1
Detector	DECADE II
E-cell	600 mV vs. HyREF
Range	50 nA/V
I cell	about 0.5 nA
ADF	0.01 Hz
Pressure	about 65 bar

Results

Working potential

In figure 3 a hydrodynamic voltammogram for Clozapine is shown. For Clozapine under the specified conditions the optimal working potential is 0.6 V.

Detection limit, repeatability and linearity

The detection limit was about 0.2 ng/mL for Clozapine using the settings listed in Table 1. The linearity of the method was determined in the concentration range of 20-100 ng/mL. The method showed a good linear detector response with correlation coefficients > 0.999. The repeatability in peak area was RSD<1% (n=6)

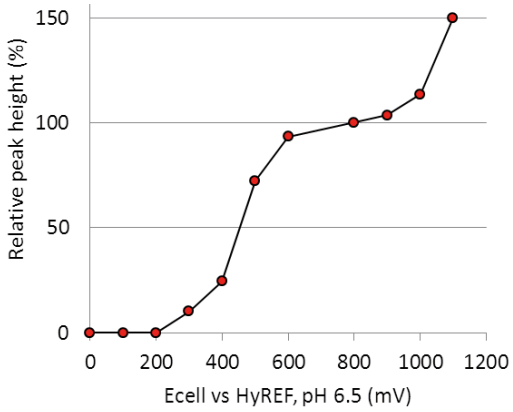

Figure 3: Hydrodynamic voltammogram of Clozapine under the LC conditions specified in Table 1.

Conclusion

Measurement conditions are presented for the analysis of Clozapine standards using an ALEXYS HPLC/ECD system. The method is reproducible and sensitive, and can be used for assay validation with real samples.

References

1. Raggi, M. A., Bugamelli, et. al., An improved HPLC-ECD method for monitoring plasma levels of clozapine and its ac-tive metabolites in schizophrenic patients, *Chromatographia*, 51 (2000) 147-153.
2. Shen, Y. L. et. al., Simultaneous determination of clozapine, clozapine N-oxide, N-desmethylclozapine, risperidone, and 9-hydroxyrisperidone in plasma by high performance liquid chromatography with ultraviolet detection, *Anal. Chim. Acta,* 460 (2002) 201-208.
3. VT-03 flowcell user manual, Antec, pn 110.0010

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an auto sampler with sample cooling option.

Ordering information

180.0035W	ALEXYS Analyzer – cooled
116.4320	SenCell 2 mm GC HyREF

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