



CLOZAPINE

THE MOST RELIABLE LC-EC APPLICATIONS FOR PHARMACEUTICAL & BIOTECH ANALYSIS EVER FORMULATED

Aminoglycosides

Amikacin
Framycetin
Gentamicin
Kanamycin
Lincomycin
Neomycin
Spectinomycin
Tobramycin

PET imaging tracer

FDG

Macrolide antibiotics

Azithromycin
Azaerythromycin
Clarithromycin
Erythromycin
Roxithromycin

Bioanalysis of pharmaceuticals

Artemisinin
Dihydro-artemisinin
Artemether
Etoposide
8-OH-DPAT
mesna BNP7787
Vincristine
Risperidone
Olanzapine
Clozapine

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 pre-treatment 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.

- Electrochemical detection of antipsychotic drugs
- Wall-jet flow cell with HyREF™ (Pd/H₂) electrode
- Reproducible & sensitive

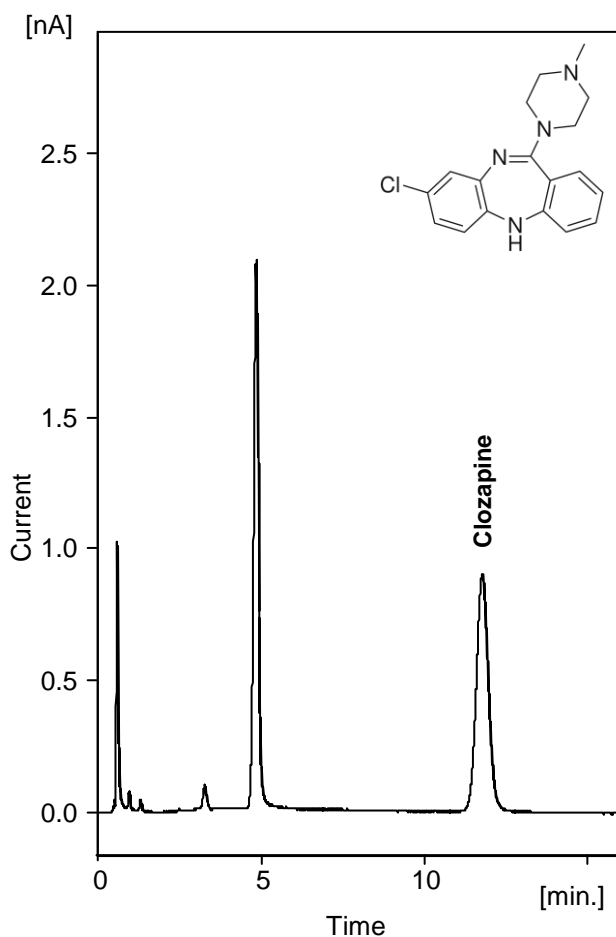


Fig. 1 Analysis of a 50 ng/mL Clozapine standard in mobile phase. Measurement conditions as given in Table 1. The peak at 5 min is from 50 ng/mL Olanzapine which was also mixed in this standard.

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 2 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)

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the DECADE II detector and ALEXYS LC-ECD system. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

for a 50 ng/mL Clozapine standard.

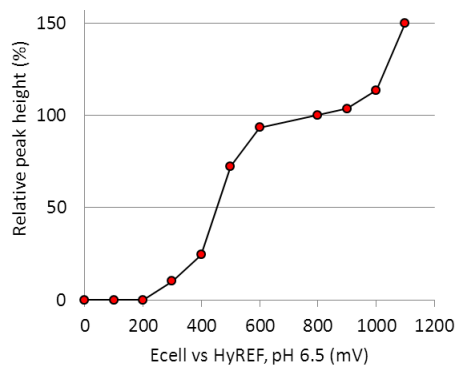


Fig. 2. Hydrodynamic voltammogram of Clozapine under the LC conditions specified in Table 1.

References

- Raggi, M. A., Bugamelli, et. al., An improved HPLC-ECD method for monitoring plasma levels of clozapine and its active metabolites in schizophrenic patients, *Chromatographia*, **51** (2000) 147-153.
- 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.
- VT-03 flowcell user manual, Antec, pn 110.0010

Recommendation

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

PART NUMBERS AND CONFIGURATION

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