RISPERIDONE

INTRODUCTION

Risperidone (trade name Risperdal®) is an atypical antipsychotic drugs belonging to the chemical class of benzisoxazole derivatives. It is used for the treatment of schizophrenia, bipolar disorder and autism. Therapeutic drug monitoring of Risperidone is advisable under some conditions [1]. Risperidone can be detected in plasma as well as saliva after sample pre-treatment [2]. Different HPLC analysis methods have been developed for separation in combination with electrochemical detection [2-5]. These methods have all described a set-up that uses coulometric flow cells for detection.

In this application note the analysis of Risperidone (proof of principle based on standards) is demonstrated using an ALEXYS LC-ECD system in combination with an amperometric wall-jet flow cell.

Electrochemical detection of antipsychotic drugs
Wall-jet flow cell with HyREF™ (Pd/H₂) electrode
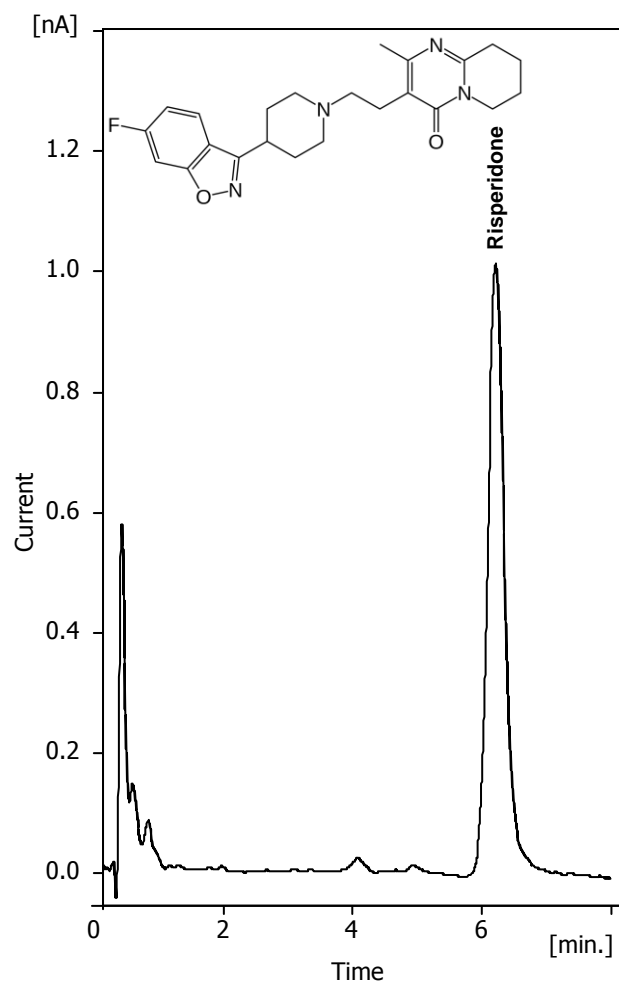Reproducible & sensitive
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Fig. 1 Analysis of 50 ng/mL Risperidone standard in mobile phase. Measurement conditions as given in Table 1.

Method

For the separation of Risperidone different conditions have been reported in literature: the use of cyano columns [3, 4], chiral column [5] and C18 column [2] with a mobile phase based on phosphate buffer at pH 6.2-6.8 and 26-90% methanol and/or acetonitrile. Risperidone standards in the analytical relevant range of 0.3-100 ng/mL were prepared in mobile phase and used to assess linearity, repeatability, detection limit and longer term response.

Conditions

In Table 1 the conditions are listed that were used to measure the reported results unless otherwise stated. As this application note demonstrates the analysis of Risperidone using amperometric detection based on standards (proof of principle), it is not necessarily a set of recommended conditions for real samples. For real sample it might be necessary to optimize the LC conditions for separation. The experiments in this application note were performed using a 1 mm ID C18 column, but the described method can be easily up scaled to standard bore LC (2 – 4.6 mm ID columns) to handle larger sample volumes.

Table 1

Conditions	
Mobile phase	Phosphate buffer 10 mM set to pH 6.5, 50% methanol
Column	C18, 50 x 1 mm ID, 3 µm particle size
Flow rate	100 µL/min
Injection volume	5 µL
Needle wash	100% acetonitrile
Temperature	35 °C
Flow cell	SenCell 2 mm GC HyREF, spacing position 1
Detector	DECADE II
E-cell	1000 mV vs. HyREF
Range	5 nA/V
I cell	about 8 nA
ADF	0.01 Hz
Pressure	About 180 bar

Results

Working potential
Risperidone is difficult to oxidize and requires a relatively high working potential. A hydrodynamic voltammogram (Fig. 2) was constructed to determine the optimal working potential. Based on signal-noise ratio, the optimal working potential was determined to be 1 V vs HyREF at pH 6.5.

Stabilization
After installation of a polished flow cell, or after electrochemical cleaning using a cleaning pulse [6], the LC-ECD system requires

at least 12 hours of stabilization (Fig. 3). After stabilization the chromatograms of the first injection should be discarded.
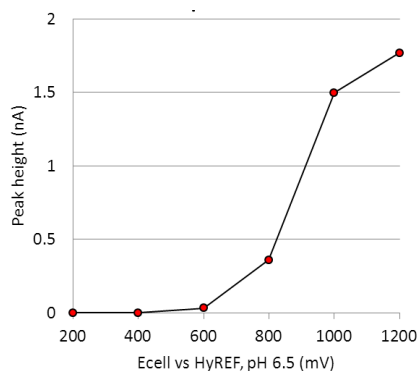

Fig. 2. Hydrodynamic voltammogram for a Risperidone standard. Mobile phase composition as given in Table 1 but with 25% acetonitrile and 25% methanol as modifier.
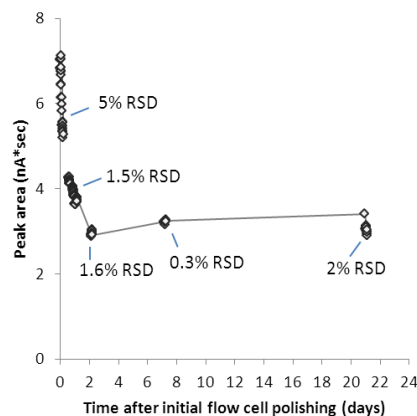

Fig. 3. Long term stability of the response of Risperidone (standard of 50 ng/mL) after polishing of the flow cell working electrode at day 0. In the gaps between days 2, 8 and 21 no analyses were performed on the system, and the system was kept standby with the cell on at 1/5th of the flow rate compared to analysis conditions. RSD values given are calculated for peak area of 6 subsequent chromatograms.

Carry-over
To minimize carry-over of Risperidone between injections pure acetonitrile was used as a wash solvent and a wash with 250 µL acetonitrile was performed after every injection.

Detection limit, repeatability and linearity
The detection limit (S/N=3) was about 0.5 ng/mL for Risperidone 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 > 0.998. The repeatability in peak area was RSD $\leq$ 2% (n=6) for a 50 ng/mL Risperidone standard in a sufficiently stabilized system.

References

1. Seto K, Dumontet J, Ensom M.H., Risperidone in schizophrenia: is there a role for therapeutic drug monitoring?, Ther Drug Monit., **33** (2011) 275-83.

2. Saracino M.A, de Palma A, Boncompagni G, Raggi, Analysis of Risperidone and its metabolite in plasma and saliva by LC with coulometric detection and a novel MEPS procedure, Talanta, **81** (2010) 1547-1553

3. Aravagiri M., Marder S. R., van Putten T., Midha K. K., Determination of risperidone in plasma by high-performance liquid chromatography with electrochemical detection: Application to therapeutic drug monitoring in schizophrenic patients, *Journal of pharmaceutical sciences*, **82** (1993) 447-449

4. Le Moing, J. P., Edouard S., Levron J. C., Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *Journal of Chromatography B: Biomedical Sciences and Applications*, **614** (1993) 333-339.

5. Locatelli, I., Mrhar, A., Grabnar, I., Simultaneous determination of risperidone and 9-hydroxyrisperidone enantiomers in human blood plasma by liquid chromatography with electrochemical detection, *Journal of pharmaceutical and biomedical analysis*, **50** (2009), 905-910.

6. VT-03 flow cell 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.

PART NUMBERS AND CONFIGURATION	
180.0035C	ALEXYS Analyzer – cooled
116.4320	SenCell 2 mm GC HyREF

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