

Feb 02, 2025

ALEXYS Neurotransmitter Analyzer for Monoamines and their Acidic Metabolites

DOI

<https://dx.doi.org/10.17504/protocols.io.q26g7m9m1gwz/v1>

Alexandria White¹

¹Emory University



Alexandria White

Emory University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.q26g7m9m1gwz/v1>

Protocol Citation: Alexandria White 2025. ALEXYS Neurotransmitter Analyzer for Monoamines and their Acidic Metabolites. protocols.io <https://dx.doi.org/10.17504/protocols.io.q26g7m9m1gwz/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working.

Created: January 30, 2025

Last Modified: February 02, 2025

Protocol Integer ID: 119431

Keywords: ASAPCRN, acidic metabolites the alexys neurotransmitter analyzer, alexys neurotransmitter analyzer, analysis of neurotransmitter, microdialysis sample, neurotransmitter, monoamine, as110 autosampler, electrochemical detection, cerebrospinal fluid, micro volume sample handling, acidic metabolite, ecd setting

Funders Acknowledgements:

Aligning Science Against Parkinson's (ASAP)

Grant ID: ASAP-020527

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

The ALEXYS Neurotransmitter Analyzer using UHPLC and electrochemical detection (ECD) has been applied for the analysis of neurotransmitters in microdialysis samples, cerebrospinal fluid (CSF) and brain tissue homogenates. HPLC and ECD settings are optimized for different target compounds with respect to selectivity and detection sensitivity. The system applies a DECADE Elite ECD with a SenCell, a powerful combination for the best possible detection limits. The AS110 autosampler facilitates micro volume sample handling (few microliters), in a dedicated injection method. Detection limits are in the range of 0.1 - 0.5 fmol on column (below 100 pmol/L in less than 10 μ L sample) and repeatability is better than 2% RSD for most components.

Materials

ALEXYS Neurotransmitter Analyzer:

- The ALEXYS Neurotransmitter Analyzer (Antec, Zoeterwoude, the Netherlands) for analysis of monoamines and metabolites consists of an OR 110 degasser unit with pulse damper(s), LC 110S pump(s), a DECADE Elite electrochemical detector, Clarity chromatography software of DataApex (Prague, The Czech Republic) and an AS 110 autosampler (other injector options are a manual injector and on-line coupling to a micro-dialysis experiment).
- A SenCell flow cell with a 2 mm glassy carbon working electrode and a sub-2µm particle 50 or 100 mm length 1.0 mm ID separation column are bundled in the additional application specific 'ALEXYS Monoamine kit' (see ordering information). Other kits are available as well, such as kits for acetylcholine, GABA and glutamate [3, 4].

Ordering information

A	B
ALEXYS Neurotransmitter Analyzer for Monoamines and metabolites	
180.0091E	ALEXYS Neurotransmitters Analyzer
191.0035UL	AS 110 autosampler UHPLC cool 6p

Application specific hardware kits

A	B
Parts in ALEXYS Monoamines kit (180.0502)	
116.4120	SenCell with 2 mm GC WE and sb REF
250.1163	Acquity UPLC BEH C18, 1.7 µm, 1 × 100 mm

A	B
Parts in ALEXYS Monoamines kit, 50 mm (180.0503)	
116.4120	SenCell with 2 mm GC WE and sb REF
250.1160	Acquity UPLC BEH C18, 1.7µm, 1 × 50mm

Sample preparation

- 1
 - Before injection, sample preparation should be applied to produce a sample that is relatively free of interferences to prevent damage like clogging of the system or column.
 - Another consideration to treat a sample is to prevent degradation of the components of interest if it will not be analyzed immediately after collection.
 - These are the treatment advises for different samples:

- 2 **Microdialysate samples**

These samples are relatively clean and can be injected in the system without the need for filtering or other treatment. However, to prevent degradation of the monoamines, acidification with or without an anti-oxidant is most often applied to the sample [5, 6]

- 3 **Brain homogenate samples**

Preparation of a sample from brain tissue usually consists of homogenization in a dilution of perchloric acid, followed by a centrifugation step to remove debris [5, 7].

- 4 **Cerebrospinal fluid**

- These sample are relatively more complex compared to microdialysis samples, and in literature it can be found that such samples either are not processed before injection, or they are acidified and centrifuged [8], or acid/anti-oxidant mix added before injection [5].
- We highly recommend to apply at least a centrifugation or filtration step before injection to remove particles: sub-2 micron columns have a higher risk of clogging compared to the larger particle columns as used in older research.

- 5 **Blood and urine (in clinical analysis)**

- For the analysis of catecholamines in blood or urine, complete SPE work-up kits are commercially available (e.g. at Chromsystems or BioRad).
- Such samples, however, have a clinical/diagnostics background, and the details are covered in another Antec application note [9].

Injection

- 6
 - A dedicated and reproducible injection program has been developed for the AS110 autosampler that efficiently handles small samples of only a few microliters.
 - The details are described elsewhere [10], in short the injection program works without 'loop overfill' that is usually applied in full loop injections.

- It efficiently transports only 2 μL in addition to the injection volume between air bubbles to the loop without 'wasting' any additional sample.
- 7
- Another mode of injection is the direct coupling of microdialysis to the ALEXYS using an electric valve. In principle, the continuous flow runs through an injection valve and at regular intervals a sample is injected.
 - The analyses described in this application note can be applied to such on-line microdialysis set-up. Details about this set-up have been described elsewhere [11].

Separation

- 8
- In the eighties of last century, a lot of research was done to develop and optimize the analysis of catecholamines, the precursors and metabolites, but this field is still progressing until today, see for example references [12 - 20].
 - Monoamines have a positive charge at $\text{pH} < 7$, and they can gain retention on a (neutrally charged) reversed phase column when ion-pairing agent is added to the mobile phase (Figure 2).

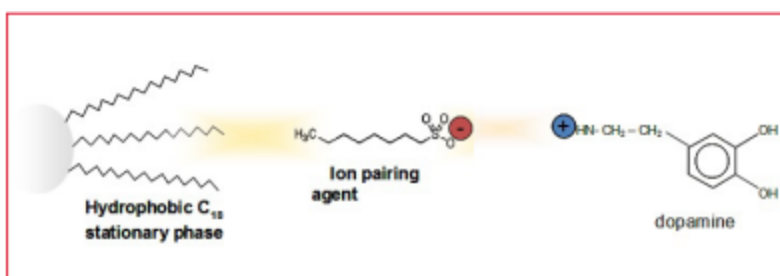


Figure 2: Schematic representation of ion-pairing principle for HPLC separation of monoamines on C18 particles

- 9
- The monoamine retention times respond to the concentration of ion pairing agent in the mobile phase (Figure 3).

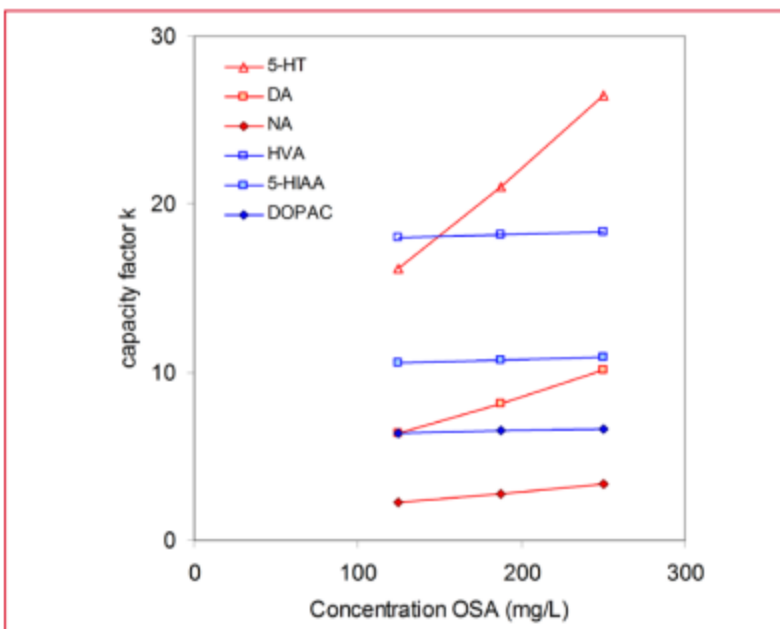


Figure 3: Effect of the ion-pairing agent octane sulfonic acid sodium salt (OSA) on retention behaviour of monoamines (red) and acidic metabolites (blue).

- 10
- The acidic metabolites have a carboxyl group with a pKa value of 4.7. They are best retained on reversed phase columns when applying a mobile phase with acidic pH.
 - When applying a neutral pH, the negative charge of the carboxyl group makes them elute in the solvent front.
 - The pH of the mobile phase therefore strongly affects the separation and retention times of acidic metabolites (fig 4.).

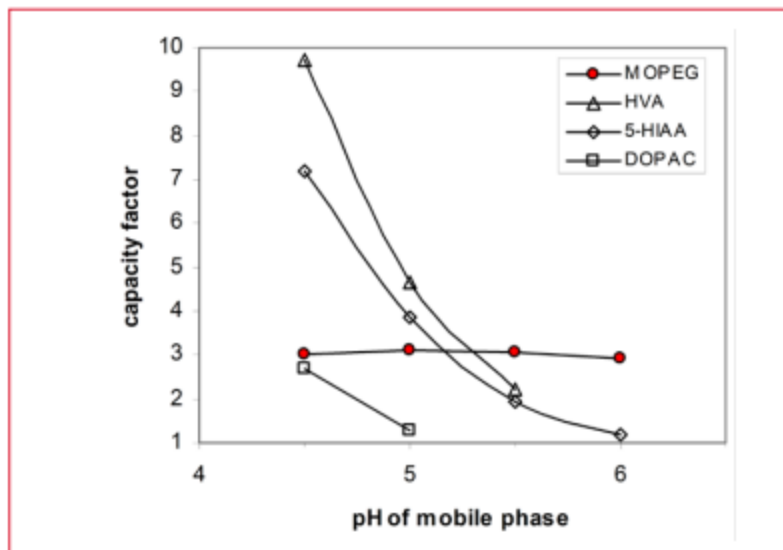


Figure 4: Effect of pH on retention behavior of molecules with a carboxyl group. For reference, the red dots indicate the retention of a molecule without a carboxyl group.

Detection

- 11
 - Monoamines and acidic metabolites are electrochemically detectable on a glassy carbon working electrode.
 - A number of excellent papers are available reporting voltammetric behavior of relevant biogenic amines and metabolites [12 - 14]. Nagao and Tanimura [14] classified the biogenic amines in four groups depending on their electrochemical behavior in a mobile phase at pH 3.6 and flow cell with glassy carbon and Ag/AgCl electrodes.
 - The four groups are: catechol compounds such as the catecholamines, DOPAC and DOPA ($E'_{1/2} = 380\text{--}500\text{ mV}$), indoles such as 5-HT and 5-HIAA ($E'_{1/2} = 480\text{--}520\text{ mV}$), vanillic compounds such as VMA, HVA and MHPG ($E'_{1/2} = 640\text{--}680\text{ mV}$) and monohydroxyphenols such as tryptophan and tyrosine ($E'_{1/2} = 870\text{ mV}$).
 - It should be noted that these given values are affected by pH (shift of about 60 mV for every pH unit), mobile phase composition and differences in glassy carbon working electrode materials.
 - It may be clear that the working potential has to be set as low as possible to ensure selectivity, but high enough to generate a clear response for the specific component(s) of interest.
 - The working potential can also be used as a tool to enhance selectivity of the method:

- 11.1 If there is only interest in the analysis of DA (and/or NA), but not 5-HT, then the working potential can be set to a lower value compared to the setting suggested in the settings table. In such case, 5-HT (and many other components) will not generate a signal.



- 11.2 For detection of the monohydroxyphenols, a relatively high potential is necessary.

Electrode activation

- 12 It is important to realize that a new or freshly polished electrode can behave differently from an electrode that is in use for a longer time. A flow cell can build up a 'history' which can result in a chromatogram with different relative peak heights compared to a new cell. However, flow cells can often be 'reinitialized' by applying an electrochemical pulse.
- 13 The HPLC is not changed, the pump is on and the usual mobile phase is applied. The detector is set to PULSE mode for about 10 min with pulse settings $E1=+1.0V$, $E2=-1.0V$, $t1=1000ms$, $t2=1000ms$, $t3=0$ and $t_s=20ms$. After 10 minutes the detector is set to DC mode at the detection potential [21].
- 14 The background current should drop below 25 nA in less than 30 min. This activation procedure can be programmed in the DECADE Elite detector and Clarity software for automated application. The pulse mode is not available in the SDC or Lite versions of the detector.

Protocol references

1. Sarre S and Michotte Y. (2007) Liquid chromatographic methods used for microdialysis: an overview. In Handbook of Microdialysis: Methods, Applications and Perspectives, Handbook of Behavioral Neuroscience, Volume 16, (Westerink BHC and Cremers TIFH, Ed.), 1st ed. pp 233-250, Academic Press, London.
2. Reinhoud NJ, Brouwer HJ, van Heerwaarden LM, Korte-Bouws GA. (2013) Analysis of glutamate, GABA, noradrenaline, dopamine, serotonin, and metabolites using microbore UHPLC with electrochemical detection. *ACS Chem Neurosci*.4(5): 888-894.
3. GABA and glutamate, histamine, amino acids. Antec (213_020)
4. Acetylcholine and choline. Antec (213_023)
5. Bicker J, Fortuna A, Alves G, Falcão A. (2012) Liquid chromatographic methods for the quantification of catecholamines and their metabolites in several biological samplesA review. *Anal Chim Acta*. 768: 12-34
6. Van Schoors J, Lens C, Maes K, Michotte Y, Smolders I, Van Eeckhaut A. (2015) Reassessment of the antioxidative mixture for the challenging electrochemical determination of dopamine, noradrenaline and serotonin in microdialysis samples, *J Chromatogr, B*, 998-999: 63-71.
7. Van Hierden YM, Korte SM, Ruesink EW, Van Reenen CC, Engel B, Korte-Bouws GAH, Koolhaas JM, Blokhuis HJ. (2002) Adrenocortical reactivity and central serotonin and dopamine turnover in young chicks from a high and low feather-pecking line of laying hens. *Physiology & Behavior*,75: 653-659
8. Koyama E, Minegishi A, Ishizaki T. (1988) Simultaneous determination of four monoamine metabolites and serotonin in cerebrospinal fluid by "high-performance" liquid chromatography with electrochemical detection; application for patients with Alzheimer's disease. *Clinical Chemistry*. 34: 680-684
9. Compilation of Clinical applications. Antec (214_007)
10. Micro volume injections. Antec (220_011)
11. On-line microdialysis of neurotransmitters. Antec (213_025)
12. Crombeen JP, Kraak JC, Poppe H. (1978) Reversed-phase systems for the analysis of catecholamines and related compounds by HPLC. *J Chromatogr.*, 167: 219-230
13. Ikarashi Y and Maruyama Y. (1985) Determination of catecholamines, indoleamines, and related metabolites in rat brain with LC with ECD. *Biogenic amines*, 2: 101-110
14. Nagao T and Tanimura T. (1989) Simultaneous determination of biogenic amines, their precursors and metabolites in a single brain of the cricket using high-performance liquid chromatography with amperometric detection. *J Chromatogr*. 496: 39-53
15. Joseph MH, Kadam BV, Risby D. (1981) Simple high-performance liquid chromatographic method for the concurrent determination of the amine metabolites vanillylmandelic acid, 3-methoxy-4-hydroxyphenylglycol, 5-hydroxyindoleacetic acid, dihydroxy-phenylacetic acid and homovanillic acid in urine using electrochemical detection, *J Chromatogr.*, 226: 361-368
16. Cheng FC and Kuo JS. (1995) Review, HPLC analysis with electrochemical detection of biogenic amines using microbore columns. *J Chromatogr., B: Biomed. Sci. Appl.*, 665: 1-13
17. Nguyen AT, Aerts T, Van Dam D, De Deyn PP. (2010) Biogenic amines and their metabolites in mouse brain tissue: Development, optimization and validation of an analytical HPLC method. *J Chromatogr, B*, 878: 3003-3014
18. Zhang J, Liu Y, Jaquins-Gerstl A, Shu Z, Michael AC, Weber SGJ. (2010) Optimization for speed and sensitivity in capillary high performance liquid chromatography. The importance of column diameter in online monitoring of serotonin by microdialysis. *J Chromatogr. A*, 1251: 54-62



19. Liu Y-S, Zhang J, Xu X-M, Zhao MK, Andrews AM, Weber SG. (2010) Capillary ultrahigh performance liquid chromatography with elevated temperature for sub-one minute separations of basal serotonin in submicroliter brain microdialysate samples. *Anal. Chem.* 82(23): 9611-9616
20. Parrot S, Neuzeret PC, Denoroy L. (2011) A rapid and sensitive method for the analysis of brain monoamine neurotransmitters using ultra-fast liquid chromatography coupled to electrochemical detection. *J Chromatogr, B*, 879: 3871– 3878
21. Sencell user manual. Antec (116_0010)
22. Šlais K and Kouřilová D. (1983) Minimization of extra-column effects with microbore columns using electrochemical detection, *J Chromatogr. A*, 258: 57-63
23. Dual cell control for improved selectivity. Antec (213_018)