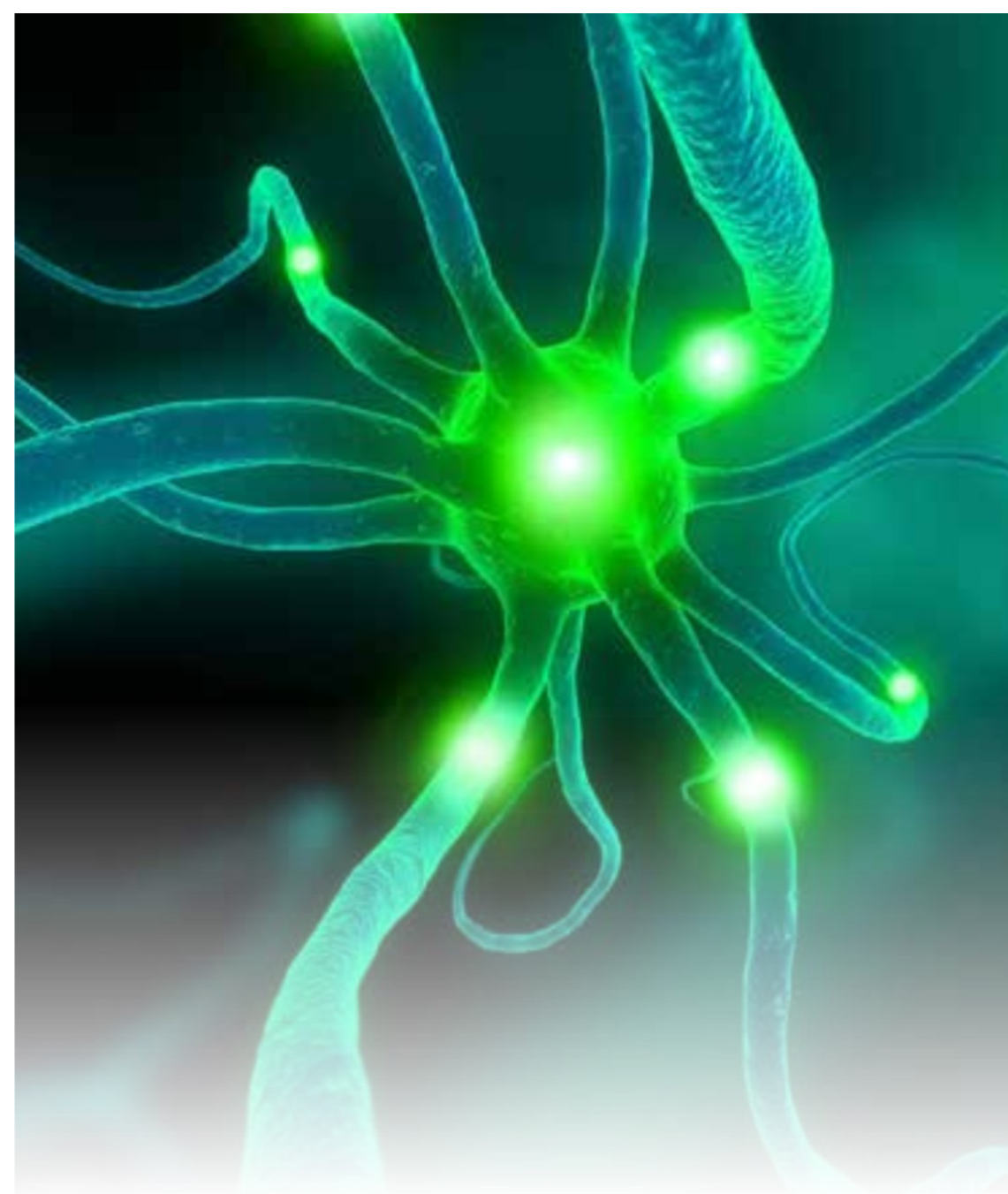


# UHPLC ALEXYS Neurotransmitter Analyzer for sensitive detection of GABA & Glutamate, Histamine, LNAAs and other Amino Acids



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## ALEXYS Neurotransmitter Analyzer

A new versatile UHPLC ALEXYS Neurotransmitter Analyzer based on the DECADE Elite detector with SenCell has been developed. This analyzer is based on a flexible and scalable approach to offer an analysis solution for multiple different neurotransmitter applications (monoamines and acidic metabolites, amino acid neurotransmitters and acetylcholine).



Fig 1. ALEXYS Analyzer with on the fore ground (right side) the SenCell and DECADE Elite electrochemical detector.

A fast and sensitive method is presented for the analysis of the amino acid neurotransmitters GABA and Glutamate in microdialysates based on the new ALEXYS neurotransmitter analyzer (see figure 1). Separation and detection is achieved using a single sub-2 µm particle column and automated pre-column derivatization with o-phthalaldehyde (OPA), respectively. A step-gradient is used for clean-up of late eluting amino acid neurotransmitters present in microdialysate samples at the end of the run. With this approach excellent detection sensitivity can be achieved with minimal sample consumption. Other Amino Acids e.g. Histamine, and Large Neutral Amino Acids (LNAAs: Tyr, Val, Met, Orn, Leu, Ile, Phe, Lys, Trp) can be analyzed too using this method.

## Method

- Automated odorless in-needle OPA-sulphite derivatization.
- Small sample use per analysis: 5 µL only (injection volume 1.5 µL)
- Fast and efficient separation using sub-2 µm particle column
- Post separation step-gradient to eliminate late eluting components
- Analysis time of 16 minutes per sample (GABA and Glu)

Conditions for GABA-Glu analysis	
Column	Acquity UPLC HSS T3 1.0 x 50 mm column, 1.8 µm
Pre-column filter	Acquity in-line filter kit
Mobile phase A (separation)	50 mM phosphoric acid, 50mM citric acid, 0.1 mM EDTA, pH3.28, 2% methanol, 1% Acetonitrile
Mobile phase B (post-separation)	40% Mobile phase A: 60% Acetonitrile
Flow rate	200 µL/min
Temperature	40 °C (separation and detection)
Vinjection	1.5 µL full loop injection as part of automated in-needle derivatization user defined program
Total sample use	5 µL
Flow cell	SenCell with GC WE and sb REF AST 1
Ecell	V= 850 mV vs Ag/AgCl (S8)
Range	50 nA/V
ADF™	Off (Glu), 0.01 Hz (for GABA, set at t= 6.20 min)

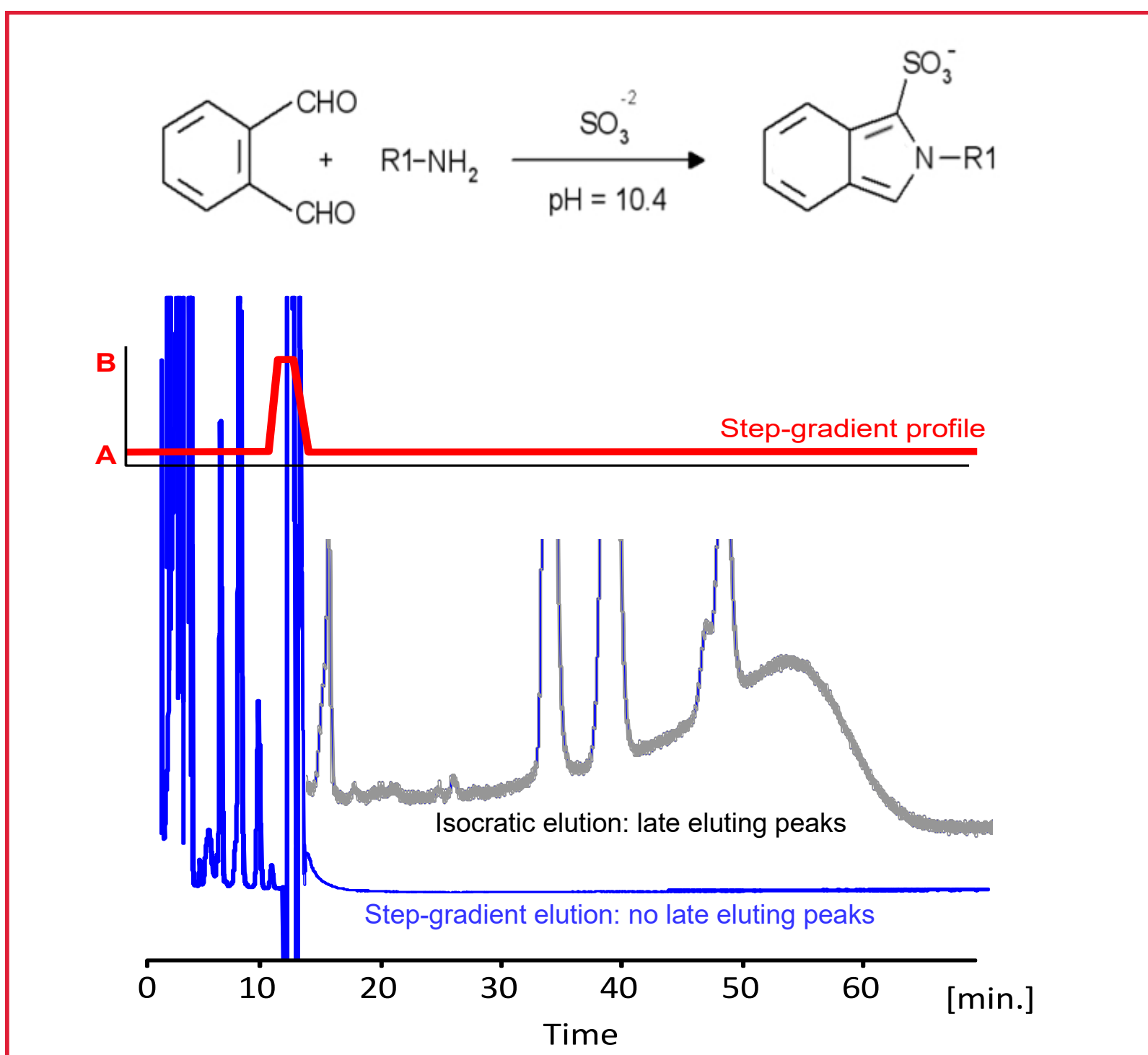


Fig 2. Top: derivatization reaction with OPA/Sulphite. Bottom: step gradient profile and clean-up of late eluting amino acids.

## Linearity, repeatability and detection limits

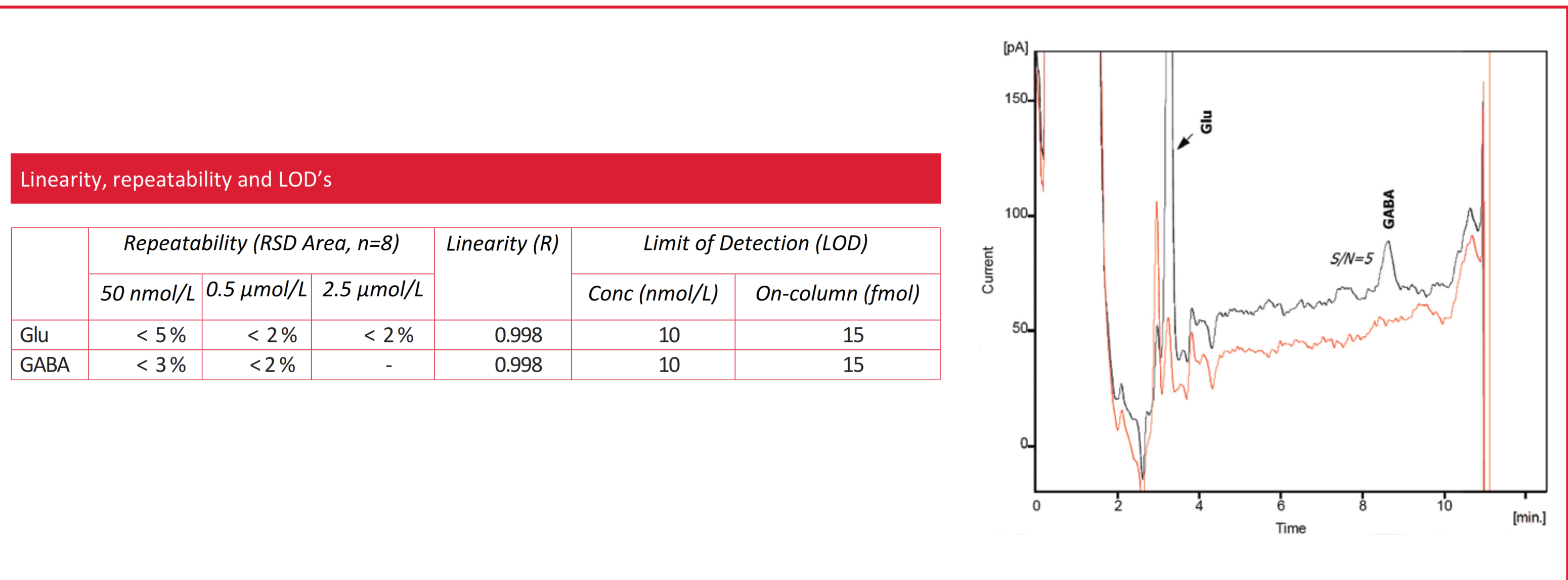


Fig 3. Left: Linearity, repeatability and LOD's. Right: Overlay of chromatograms of a blank (ringer) and a mix of 200 nmol/L Glu and 20 nmol/L GABA in Ringer.

## Automated mobile phase optimization

The Clarity chromatography data system in combination with an ALEXYS system with two pumps can be used for automated optimization of the mobile phase composition to achieve optimal separation of GABA, Glu and other amino acids.

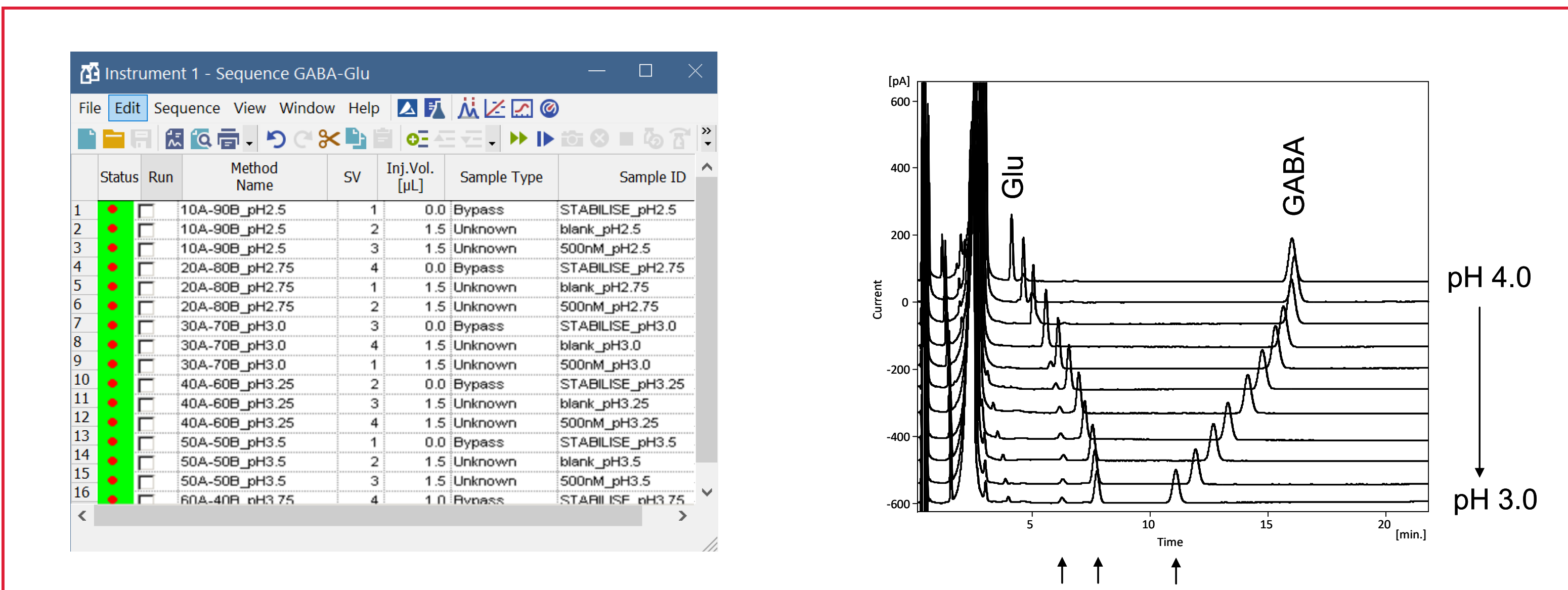


Fig 4. Left: example of a sequence in the Clarity software for automated mobile phase optimization. Right: Overlay of chromatograms obtained with different pH of the mobile phase (pH 3 – 4).

## GABA, Glu and other amino acids

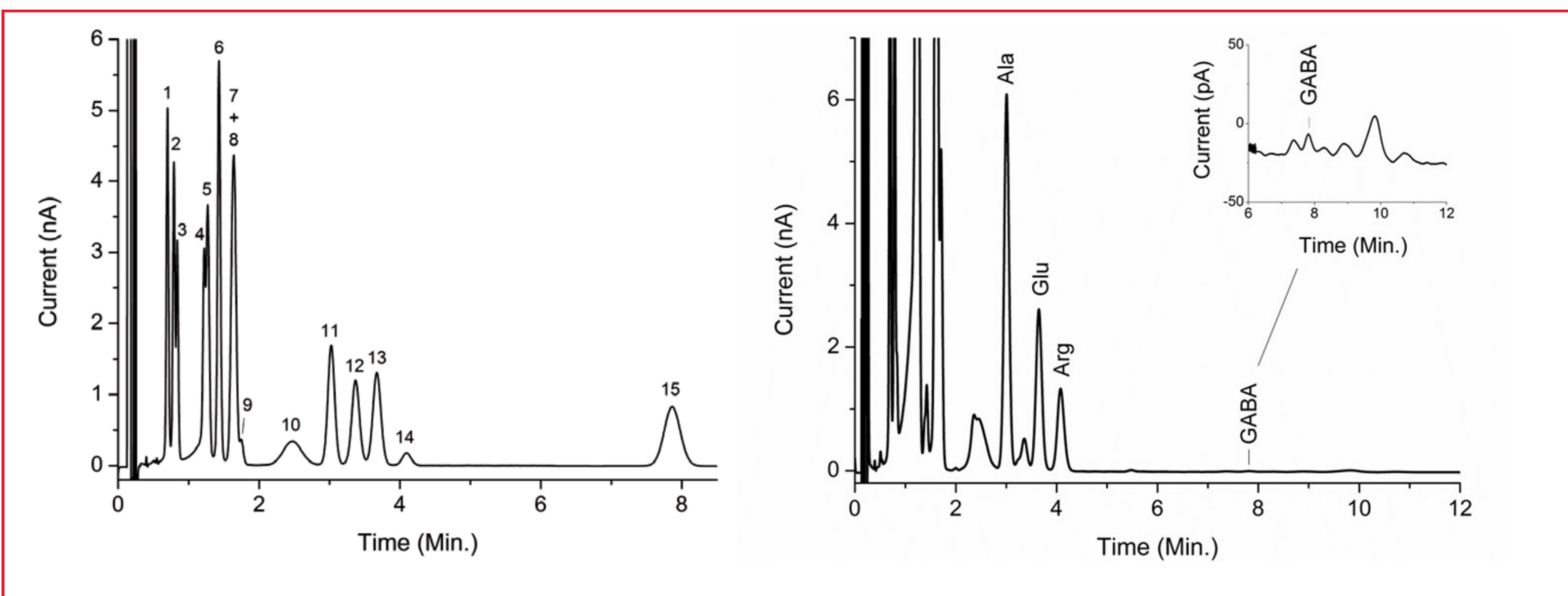


Fig. 5. Left: Chromatogram of a 2.5 µmol/L standard of 14 amino acids in water, derivatised with ortho-phthalaldehyde (OPA): (1) Serine, (2) Taurine, (3) Asparagine, (4) OPA, (5) Glycine, (6) Histidine, (7) Aspartate, (8) Glutamine, (9) Cystine, (10) Trans 4-hydroxy-L-Proline, (11) Alanine, (12) Citrulline, (13) Glutamate, (14) Arginine and (15) GABA. Right: Chromatogram of a rat prefrontal cortex dialysate sample. Insert: zoom-in on GABA peak.

## Histamine

Another example is the analysis of the biogenic amine Histamine using the ALEXYS Neurotransmitter Analyzer. Histamine is considered as one of the most important mediators of allergic reactions and inflammations. A slightly modified mobile phase (pH 6.0) is used to separate Histamine.

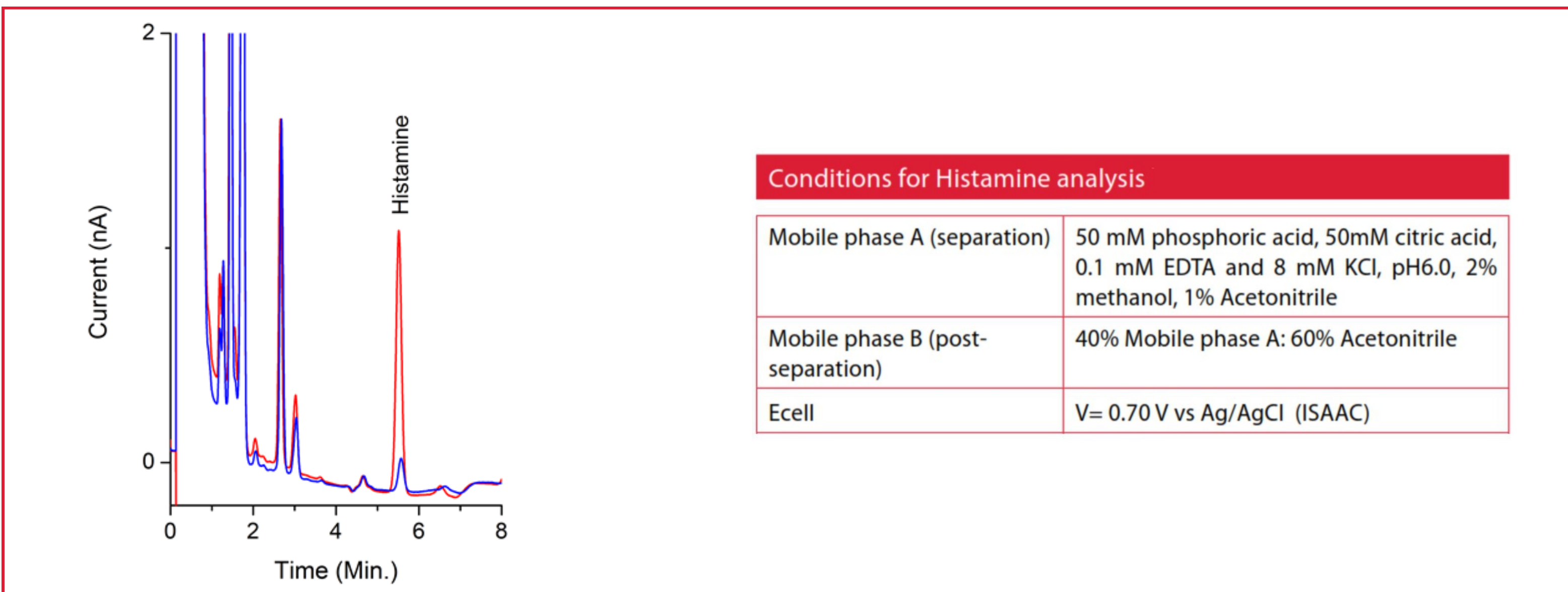


Fig 6. Left: Analysis of the Histamine release in RBL-2H3 (mast cell model) after an allergen trigger. Chromatogram A (blue curve): Histamine level in blank (solution with RBL-2H3 cells before exposure to allergen). Chromatogram B (Red curve): Histamine level after exposure to DNP-BSA allergen. Chromatograms courtesy of Mrs. Gerdien Korte-Bouws, Department of Pharmaceutical Sciences, division of Pharmacology, University of Utrecht, The Netherlands. Right: conditions.

## Large Neutral Amino Acids (LNAAs)

LNAAs (Tyr, Val, Met, Orn, Leu, Ile, Phe, Lys, Trp) can also be measured with this method using a mobile phase (pH 4.5) with a larger contents of organic modifier.

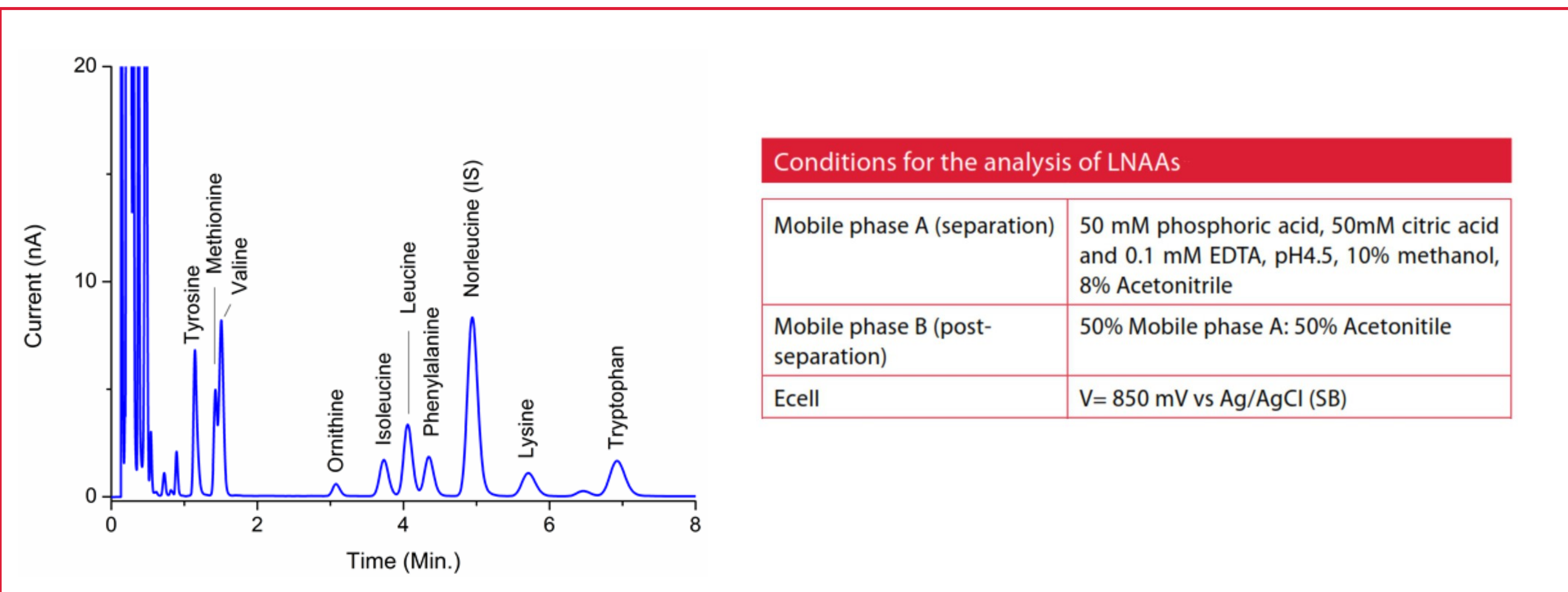


Fig 7. Left: Analysis of extracted chicken plasma. Chromatogram courtesy of Mrs. Gerdien Korte-Bouws, Department of Pharmaceutical Sciences, division of Pharmacology, University of Utrecht, The Netherlands. Right: conditions.

## Conclusions

- Automated** - Fully automated in needle sample derivatization
- Fast** - 16 min analysis time
- Sensitive** - LOD for GABA <10nM using only 1.5 µL sample (15 fmol on-column)
- Versatile** - GABA, Glu, Histamine, LNAAs and other amino acids
- Rodent saving** - More information from less samples, reduces the number of assays and lab animals involved.

