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CLINICAL

Application Notes

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Analysis of uric acid and related compounds in urine samples

empresa innovadora de base tecnológica reconocida por ANCES

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» INTRODUCTION

Clinical analysis is demanding new and competitive analytical methods. Conventional systems require a large amount of time and money for reagents and instruments, which is not compatible with the enormous number of analyses that are needed in daily life.

Clinical analysis of urine is one of the most important inspections for medical diagnosis. **Uric acid** (UA) is the final breakdown product of dietary or endogenous purines. Clinical studies have shown that monitoring UA levels in urine and blood serum can be used to diagnose several diseases, such as *hyperuricemia* and *gout*.

Microfluidic electrophoresis platforms enable the possibility of *monitoring UA* levels as well as the separation of interference compounds such as epinephrine, L-DOPA, ascorbic acid, acetaminophen, xanthine, theophylline or caffeine coming from endogenous or exogenous sources.

Electrochemical approaches also allow the direct detection of uric acid and related compounds. So, conventional methodologies that are temperature-dependent, expensive and require labile reagents (enzymes) are improved.

Therefore, **microfluidic systems** bring novel methodologies for fast, inexpensive and high throughput **urine analysis**.

» EXPERIMENTAL

Samples: Uric acid and related compounds standard solutions.
Urine samples 10-fold diluted in the buffer solution.

Injected sample: < 100 pL.

Instrumentation: MicruX® HVStat / iHVStat.
Holder DC series.

Microfluidic device: SU8/Pyrex microchips with integrated Pt electrodes (*MCE-SU8-Pt001T*).

Conditioning: 0.1 M NaOH – 30 min.
Deionized water – 15 min.
Buffer solution – 10 min.

» RESULTS

Microfluidic electrophoresis system has been used in the separation and detection of uric acid (UA) as well as related compounds such as acetaminophen (APAP), epinephrine (EP), ascorbic acid (AA) and *p*-aminophenol (*p*AP).

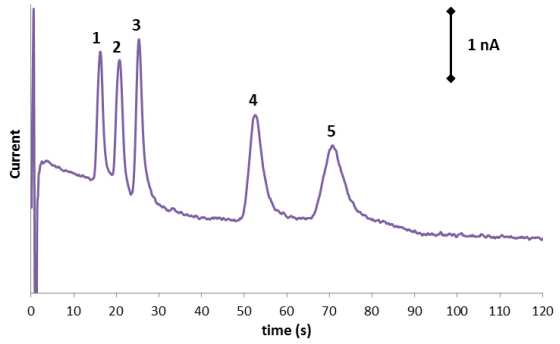

***Figure 1**. Electropherogram for a sample mixture of **(1)** EP 50 µM, **(2)** pAP 50 µM, **(3)** APAP 100 µM, **(4)** UA 125 µM and **(5)** AA 250 µM using a SU-8/Pyrex microchip with integrated Pt film end-channel detector. Conditions: V_{inj} = +750 V, t_{inj} = 3 s, V_{sep} = +1000 V, E_d = +0.8 V (vs. Pt) and 20 mM MES, pH = 6.0 as running buffer.*

The separation of the five compounds in the experimental conditions is accomplished in less than **90 s** (***Figure 1***).

Uric acid can be determined in human urine samples from healthy patients. The sample is evaluated using the *standard additions method* in order to avoid any matrix effect and get a better precision.

Typical reference value for the uric acid in urine is 250 – 750 mg/24h (1.49 – 4.5 mmol/24h) for a healthy individual.

The average UA amount found in the urine sample of the healthy volunteer was *2.1 ± 0.5 mmol* (***Figure 2***).

Severo Ochoa Building. Floor -1 · Room 4 & 6. Julián Clavería, s/n 33006 · Oviedo (Asturias). Spain.
T/F +34 984 151 019 · E info@micruxfluidic.com · W www.micruxfluidic.com

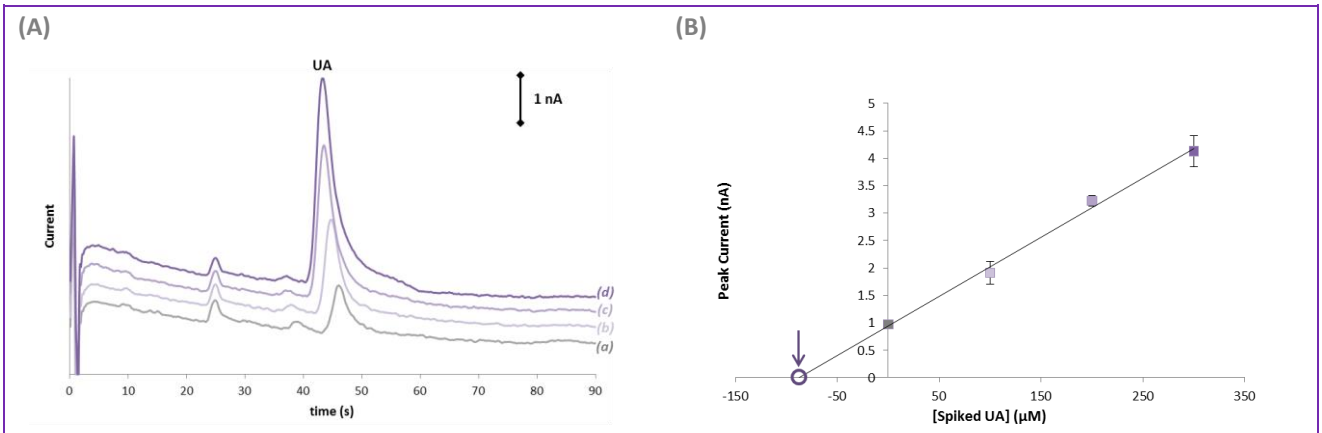

Figure 2. ***(A)*** *Electropherograms for a 10-fold dilute human urine sample with successive additions of UA: 0 (a); 100 (b); 200 (c) and 300 µM (d).* ***(B)*** *Standard additions calibration plot. Conditions: V_{inj} = +750 V, t_{inj} = 3 s, V_{sep} = +1000 V, E_d = +0.8 V (vs. Pt) and 20 mM MES, pH = 6.0 as running buffer.*

The methodology enables the direct detection of uric acid without previous enzymatic based-reactions or other complex pretreatment. The urine sample is simply diluted in the buffer solution and transfer to the microfluidic platform.

Thus, the portable microfluidic platform can be successfully used for the separation, detection and quantification of electrochemical active compounds in biological samples (urine, serum, plasma…).

The automated analytical microfluidic system can be validated for clinical trials and currently, it is used as educational tool (*Teaching Packs*) in Universities.

Table 1. *Analytical parameters for the separation of uric acid and related compounds using a SU8/Pyrex chip with integrated Pt electrode*

	EP	*p*AP	APAP	UA	AA
» Repeatability i_p (RSD %)	4%	5%	2%	4%	3%
» Repeatability t_m (RSD %)	0.5%	0.5%	0.5%	1%	1%
» Theoretical plate number (N|m^{-1}):	32.000	47.000	67.000	63.000	47.000
» Resolution (R_s):	1.2	0.8	2.6	1.4	
» Linear range (µM):	5 – 500	5 – 500	10 – 200	20 – 400	30 – 500
» Sensitivity (pA·µM^{-1}):	46	44	17	17	9
» LOQ (µM):	5	5	10	20	30
» LOD (µM)*:	4	4	9	10	15

**Limit of detection considers a signal-to-noise ratio, S/N = 3.*
Conditions: 20 mM MES, pH = 6.0; V_{inj} = +750 V, t_{inj} = 3 s, V_{sep} = +1000 V, E_d = +0.8 V (vs. Pt). EP (50 µM), pAP (50 µM), APAP (100 µM), UA (125 µM), AA (250 µM).

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TECHNOLOGIES

Severo Ochoa Building · Floor -1 – Room 4 & 6
Julián Clavería s/n · 33006 · Oviedo (Asturias) · SPAIN

Phone/FAX: +34 984151019

E-mail: info@micruxfluidic.com
Web: www.micruxfluidic.com

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