evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **Analytical challenges of detection of Doxorubicin using separation techniques**

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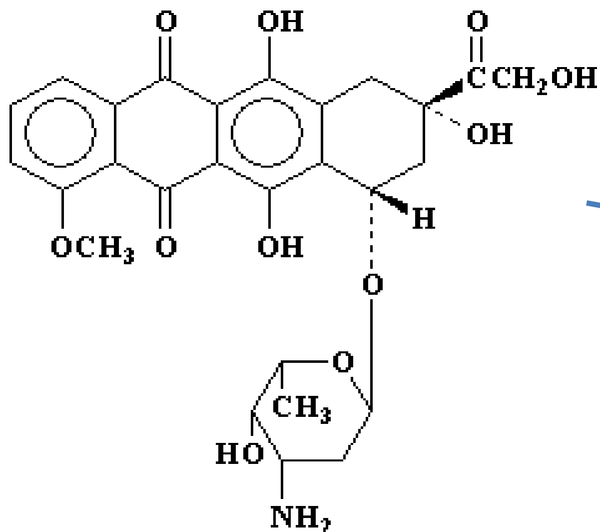
Reg.č.projektu: CZ.1.07/2.3.00/20.0148

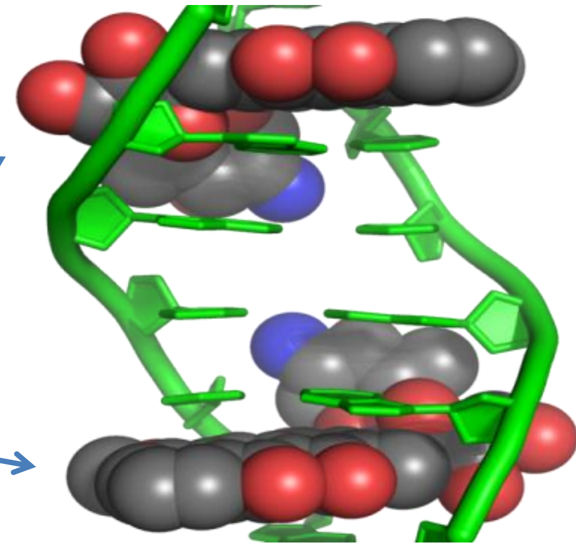
Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

LAB SYS

Doxorubicin

- **Broadband anthracycline antibiotic, firstly isolated in 1950 from bacteria *Streptomyces peucetius*.**
- **One of the most used and the most powerful natural anticancer drug.**
- **It is useful in both monotherapy and with other cytostatics as well.**
- **Mechanism of effect – intercalation into DNA.**
- **It is fluorescent active.**
- **<u>Negative side-effects</u>.**





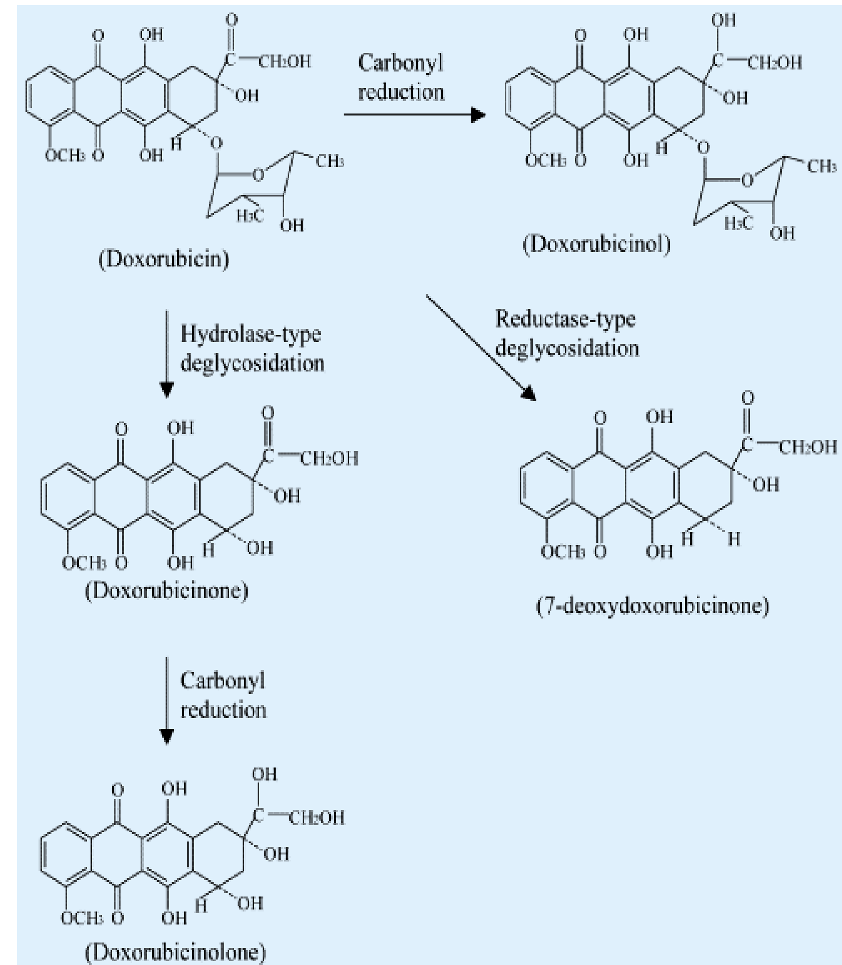
intercalation of DOXO into DNA

Negative side-effects: metabolization

- The most frequent transformation of DOXO is in liver, and excretion by primary bile.

Primary metabolite – **doxorubicinol** has clinical importance, because it shows similar effects as doxorubicin.

- **Doxorubicinol** and other metabolites – **doxorubicinon, doxorubicinolon, 7-deoxydoxorubicinon** or **7-deoxydoxorubicinolon** probably contributes into the free radicals forming and thus to higher occurrence of negative side-effects.



Analysis of intracellular doxorubicin and its metabolites by ultra-high-performance liquid chromatography

Chromatographic conditions:

High-throughput quantification of doxorubicin and its metabolites was performed using a Hitachi LaChrom ULTRA system and an **fluorescence detector**. Samples were analyzed on a **Capcell Pak C18 IF column** (**2.0 × 50 mm; particle size, 2 µm**; Shiseido Corp., Tokyo, Japan). The fluorescence detector was operated at an excitation wavelength of **470 nm** and an emission wavelength of **590 nm**. A volume of **5 µL of sample was injected** each time.

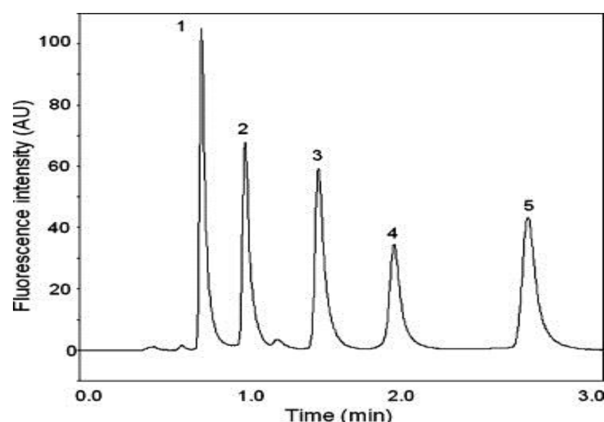


Figure: Chromatogram of doxorubicin and its metabolites. 1) doxorubicinol; 2) doxorubicin; 3) doxorubicinolone; 4) daunorubicin (internal standard); 5) doxorubicinone.

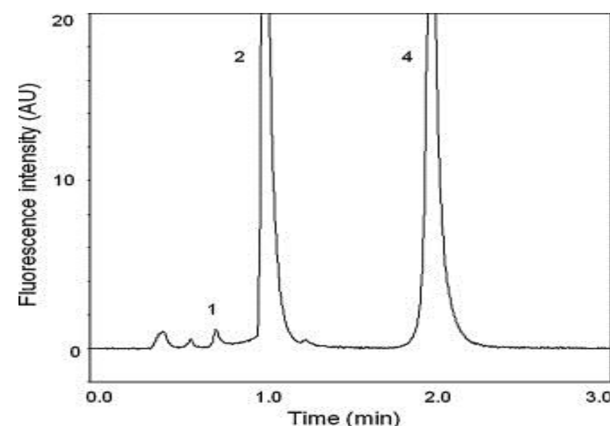


Figure: Chromatogram of cell homogenate obtained 2 h after administration of doxorubicin. HeLa cells were exposed to 10 µg/mL doxorubicin for 2 h. 1) doxorubicinol; 2) doxorubicin; 4) daunorubicin (internal standard).

Table 1
Detection limits and quantitation limits of doxorubicin and its metabolites.

Compound	Detection limit (pg/injection)	Quantitation limit (pg/injection)
Doxorubicin	5.2	17.4
Doxorubicinol	3.5	11.7
Doxorubicinolone	6.0	19.8
Doxorubicinon	7.4	24.5

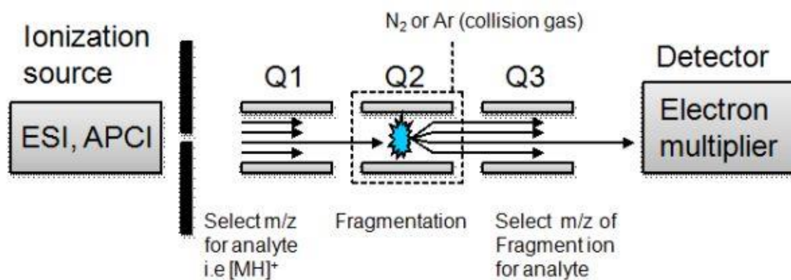
The detection and quantitation limits of doxorubicin and its metabolites were determined based on signal-to-noise ratios (3:1 for detection limits, and 10:1 for quantitation limits).

Reference:

SAKAI-KATO, K., E. SAITO, et al., Analysis of intracellular doxorubicin and its metabolites by ultra-high-performance liquid chromatography. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*.**2010**, **878**(19): 1466-1470.

Simultaneous quantification of ten cytotoxic drugs by a validated LC–ESI–MS/MS method

A **liquid chromatography separation with electrospray ionisation and tandem mass spectrometry** detection method was developed for the simultaneous quantification of ten commonly handled cytotoxic drugs in a hospital pharmacy **(cytarabine, gemcitabine, methotrexate, etoposide phosphate, cyclophosphamide, ifosfamide, irinotecan, doxorubicin, epirubicin and vincristine)**. The chromatographic separation was carried out **by RPLC in less than 21 min**, applying a gradient elution of water and acetonitrile in the presence of 0.1% formic acid. MS/MS was performed on a **triple quadrupole** in selected reaction monitoring mode (**SRM**). **lowest LOQs were between 0.25 and 2 ng mL^{-1}** for the ten investigated cytotoxic drugs; **trueness values (i.e. recovery) were between 85% and 110%**.



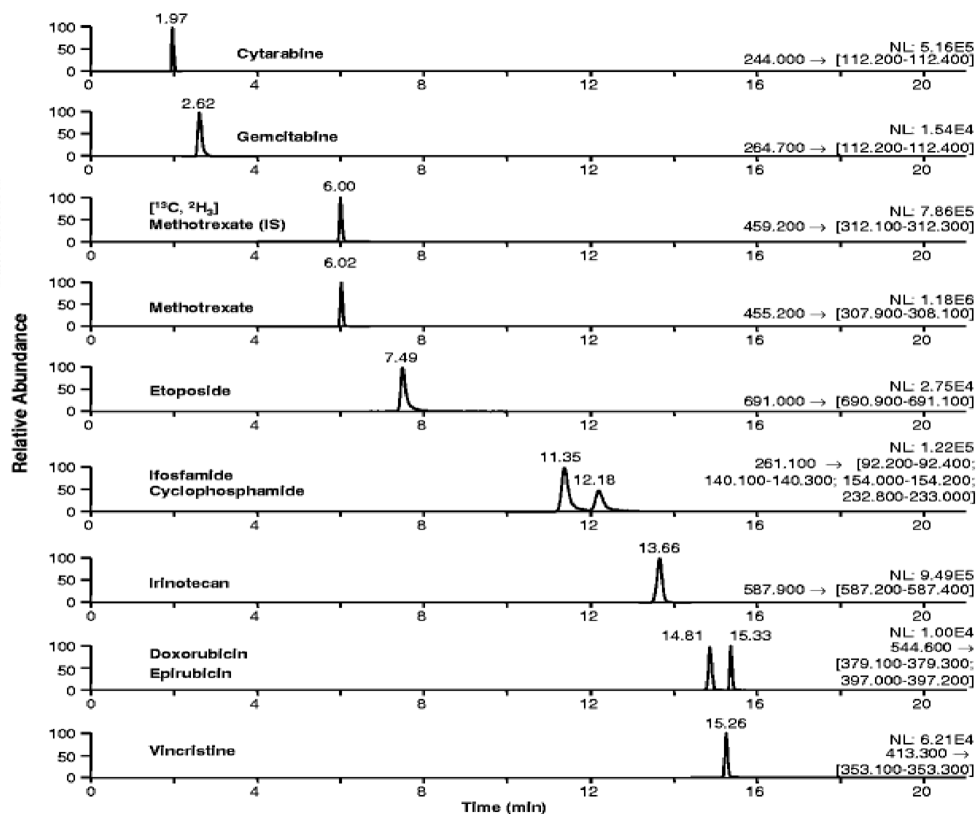


Figure: LC–MS/MS chromatogram of a sample containing ten cytotoxic drugs and the internal standard at 50 ng mL^{-1}. Column: ZORBAX SB-C18 RR 2.1 × 100 mm 3.5 µm; flow rate 200 µL min^{-1}; gradient conditions and scan events are reported reference.

Reference:

NUSSBAUMER, S., S. FLEURY-SOUVERAIN, et al., Simultaneous quantification of ten cytotoxic drugs by a validated LC-ESI-MS/MS method. *Analytical and Bioanalytical Chemistry*.2010, **398**(7-8): 3033-3042.

Separation and simultaneous detection of anticancer drugs in a microfluidic device with an amperometric biosensor

An **amperometric detection with dsDNA and cardiolipin modified screen printed electrodes** are used for the detection of anticancer drugs at the end of separation channel. **The preconcentration capacity is enhanced thoroughly using field amplified sample stacking (FASS) and field amplified sample injection (FASI)** techniques. The calibration plots are linear with the correlation coefficient between 0.9913 and 0.9982 over the range of 2-60 pM. **The detection limits** of four drugs are determined to be **between 1.2 (+/- 0.05) and 5.5 (+/- 0.3) fM**. The applicability of the device to the direct analysis of anticancer drugs is successfully demonstrated in a **real spiked urine sample**. Device was also examined for interference effect of common chemicals present in real samples.

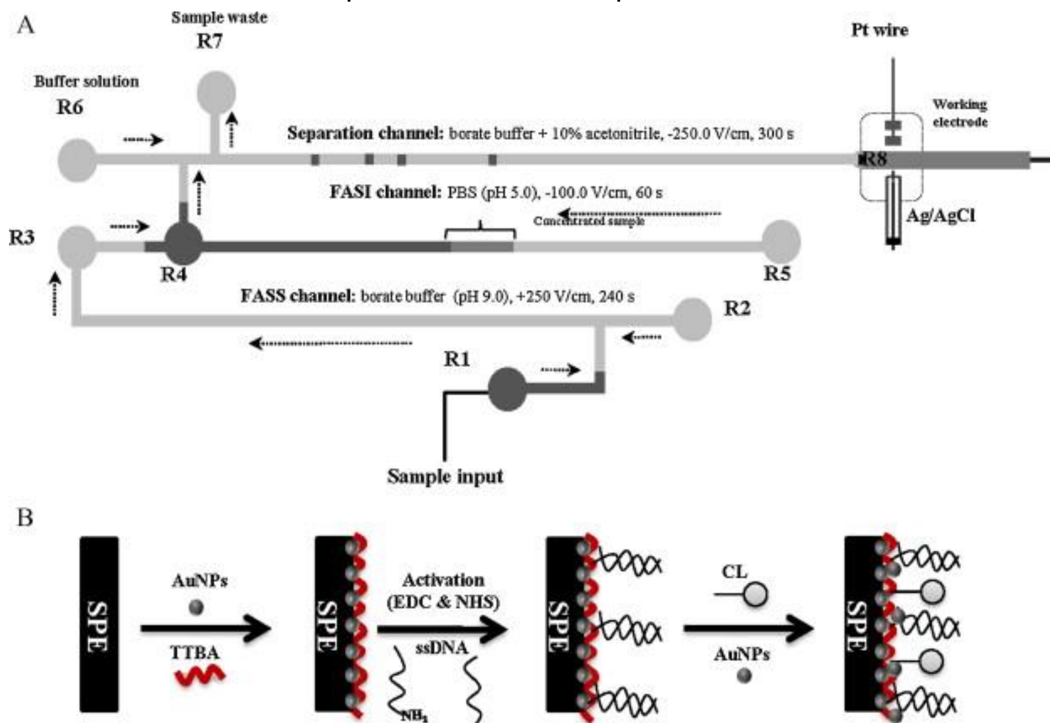


Figure: (A) Schematic diagram of the microfluidic device. (B) Schematic representation of the biosensor fabrication.

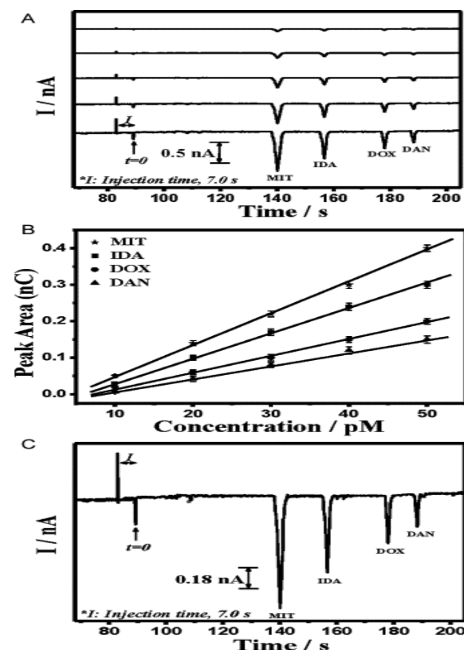


Figure: (A) Electropherograms obtained for varying concentrations anticancer drugs, (B) calibration curves obtained by plotting the signals obtained in (A) and (C) **electropherogram obtained for drugs (mitoxantrone, idarubicin, doxo, daunomycin) in urine sample**. SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs sensor probe. Detection potential, −0.7 V. Separation field strength, −2 kV; injection time, 7.0 s at −2 kV; running buffer, borate buffer +10% acetonitrile, and detection buffer 100 mM PBS (pH 7.4).

Reference:

CHANDRA, P., S. A. ZAIDI, et al., Separation and simultaneous detection of anticancer drugs in a microfluidic device with an amperometric biosensor. *Biosensors & Bioelectronics*.**2011**, **28**(1): 326-332.

Monitoring the subcellular localization of doxorubicin in CHO-K1 using MEKC-LIF: Liposomal carrier for enhanced drug delivery

Reported modified **MEKC–LIF** (Micellar electrokinetic chromatography–Laser-induced fluorescence) method is suitable for analyzing DOX in biological samples. The MEKC migration buffer, consisting of 10 mM borate, 100 mM sodium dodecyl sulfate (SDS) (pH 9.3), was found to provide an efficient and stable electrophoretic separation and analysis for DOX. **limit of detection (LOD) was calculated as 6.36 ng/mL (S/N=3)** (equivalent to 11.0 nM).

This approach was employed to compare the intracellular accumulation of DOX in CHO-K1 cells. These fractions fractions determined were pellet enriched in **nuclei**, **organelles** (mitochondria and lysosomes), and **cytosole components**, respectively, resulting from **treatment of CHO-K1 (Chinese hamster ovary line) cells with 25 µM** (equivalent to 14.5 µg/mL) of two DOX formats (in free drug form or liposomal form synthesized in current study) for different periods of time.

The most abundant DOX was found in the nuclear-enriched fraction of cells treated for 12 h and 6 h with free and liposomal DOX, respectively, providing direct evidence to **confirm the enhanced efficiency of liposomal carriers in delivering DOX into the nucleus**. **The observations presented herein suggest that subcellular fractionation followed by liquid–liquid extraction and MEKC-LIF could be a powerful diagnostic tool for monitoring intracellular DOX distribution**.

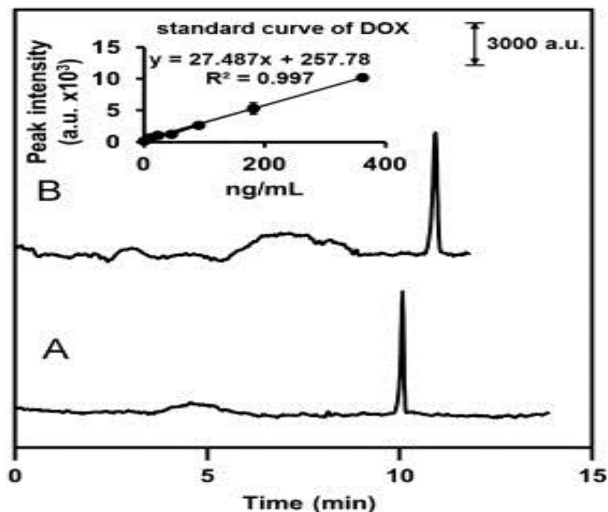


Table 1
Analytical performance of the LLE method in conjugation with modified MECK–LIF detection.

DOX (ng/mL)	Recovery (% ± SD)	Precision (%RSD)		LOD (ng/mL)
		Intraday	Interday	
181	98.2 ± 3.7	2.04	3.73	6.36
527	97.2 ± 1.6	0.98	1.60	
$n=3$				

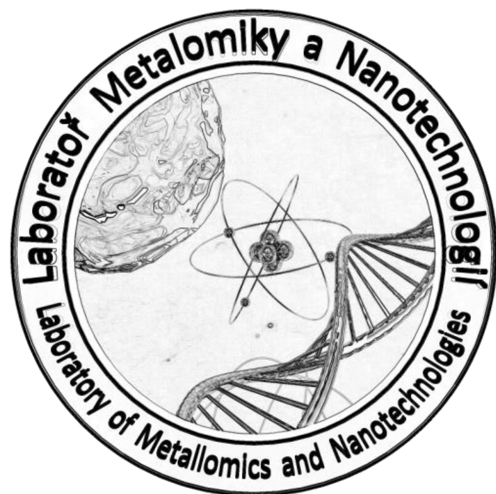
Figure: Electropherograms for (A) the standard solution of DOX, 300 ng/mL (equivalent to 517 nM), prepared in phosphate buffered saline (pH=7.4); (B) CHO-K1 cell lysate spiked with 300 ng/mL (equivalent to 517 nM) of DOX. Inset shows the standard curve for DOX. Migration buffer: 10 mM borate buffer containing 100 mM sodium dodecyl sulfate (pH 9.3), separation voltage, 25 kV.

Reference:

Ho, J.A.A., et al., *Monitoring the subcellular localization of doxorubicin in CHO-K1 using MEKC-LIF: Liposomal carrier for enhanced drug delivery.* Talanta, **2012**. **99**: p. 683-688.

Acknowledgements

- Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik CZ.1.07/2.3.00/20.0148
- Thanks to Roman Guran for assistance.



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Thank you for your attention!

Reg.č.projektu: CZ.1.07/2.3.00/20.0148

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