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# On-line column coupled isotachophoresis-capillary zone electrophoresis hyphenated with tandem mass spectrometry in drug analysis: Varenicline and its metabolite in human urine

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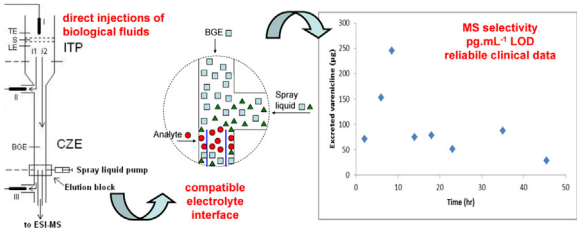
## HIGHLIGHTS

- New hyphenation was based on two-dimensional CE (ITP-CZE) and tandem MS (QqQ).
- 2D-CE mode provided on-line sample preparation (preconcentration, sample clean-up).
- ITP-CZE–ESI-QqQ combination was approved by excellent performance parameters.
- Application area involves trace (pg $mL^{-1}$) drugs in unpretreated complex matrices.
- We proposed routine clinical method for varenicline and its metabolite in urine.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A new highly advanced analytical approach, based on two-dimensional column coupled CE (ITP-CZE) hyphenated with tandem mass spectrometry (MS/MS, here triple quadrupole, QqQ) was developed, evaluated and applied in biomedical field in the present work. Capillary isotachophoresis (ITP) coupled on-line with capillary zone electrophoresis (CZE) used in hydrodynamically closed separation system was favorable for increasing the sample load capacity, increasing the analyte concentration, and removing the deteriorative highly conductive major matrix constituents. These factors considerably reduced the concentration limits of detection (cLOD) and external sample preparation (comparing to single column CZE), and, by that, provided favorable conditions for the mass spectrometry (enhanced signal to noise ratio, reproducibility of measurements, working life of MS). Here, the CZE–ESI combination provided more effective interfacing than ITP–ESI resulting in both a higher obtainable intensity of MS detection signal of the analyte as well as reproducibility of measurements of the analyte's peak area. The optimized ITP-CZE–ESI-QqQ method was successfully evaluated as for its performance parameters (LOD, LOQ, linearity, precision,

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recovery/accuracy) and applied for the direct identification and ultratrace (pg mL$^{-1}$) determination of varenicline and, in addition, identification of its targeted metabolite, 2-hydroxy-varenicline, in unpretreated/diluted human urine. This application example demonstrated the real analytical potential of this new analytical approach and, at the same time, served as currently the most effective routine clinical method for varenicline.



## 1. Introduction

Varenicline, an analog of cytisine, acts as a partial agonist with the affinity and selectivity for $\alpha_4\beta_2$ nicotinic acetylcholine receptors in the ventral tegmental area of the brain and inhibits nicotine-induced dopaminergic acivation [1]. This dual effect (afinity/activity) at the receptors helps the patients to achieve smoking cessation by reducing cravings, withdrawal symptoms and smoking satisfaction [2,3].

Varenicline (7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino [2,3-h] [3] benzazepine), as tartrate salt, was approved by the Food and Drug Administration (FDA) in the USA and by the European Medicines Agency (EMA) in 2006 under the trade names Chantix and Champix, respectively. The recommended dose of this drug is 1 mg twice daily [4,5]. Varenicline undergoes minimal metabolism. The oxidative metabolism of varenicline is not associated with the cytochrome P450 in the liver and it is primarily excreted unchanged in urine [6]. Less than 10% is excreted as metabolites – varenicline *N*-carbamoylglucuronide and 2-hydroxyvarenicline [5,7]. The elimination half-life of varenicline is approximately 24 h. Metabolism of varenicline in humans and excretion pathways of its metabolites with corresponding chemical structures are shown in Fig. 1.

Several analytical methods for the identification and quantification of varenicline and/or its (bio) degradation products in pharmaceutical and biological samples were described, such as CE-DAD and ITP-CZE-UV for tablets [8,9], voltamperometry for spiked (i.e., model) plasma [10], GC–MS for blood and urine [11] and UPLC and HPLC methods for spiked as well as real blood, plasma, serum, urine, tissue samples [6,12–18]. In case of biological samples, due to relatively very low dose of varenicline administered, a highly sensitive detection method and/or appropriate sample preparation was prerequisite for the reliable determination of the drug and its metabolite(s). All the methods, used for the determination of varenicline in the real biological samples so far, were combined with an external (off-line) sample preparation procedure such as derivatization of the analyte, SPE, LLE or deproteination in order to improve their selectivity and detection limits. Detection limits ranged from 0.1 ng mL$^{-1}$, when using a sensitive QqQ detection [13], to 500 ng mL$^{-1}$, when using a conventional DAD detection [15] in the pretreated plasma samples, while it was 50 ng mL$^{-1}$ when using DAD detection [15] in the urine sample.

To avoid an external manipulation with the biological samples that is time consuming, limited by the sample volume and increases the risk of analyte loss, the on-line sample preparation methods can be advantageously applied. Generally, these are mainly HPLC, ITP, SPE, LLE, microdialysis and ultrafiltration pretreatments on-line combined with the HPLC and CE methods [19]. Among them, the on-line combined column coupled capillary electrophoretic techniques (i.e., two different/separate columns are coupled by bifurcation block serving as an ITP-CZE interface), namely ITP–ITP, ITP-CZE, were introduced by Everaerts et al. [20] and adapted to the currently used commercial modular analyzers by Kaniansky and Marak in 90s [21]. This column coupled 2D-CE approach is a very attractive alternative to the hyphenated chromatographic techniques due to its simplicity, cost, separation efficiency, versatility, flexibility and environmental aspects. In the last decade, a great effort was made to hyphenate the two-dimensional column coupled CE separation with the MS detection in order to simplify and effectivize sample clean-up, and enhance sensitivity, analyte identification and application range. The instrumental arrangement of the column coupled ITP-CZE system allows removing the sample matrix (it is directed to the ITP-CZE interface's electrode compartment) so that it cannot migrate to the

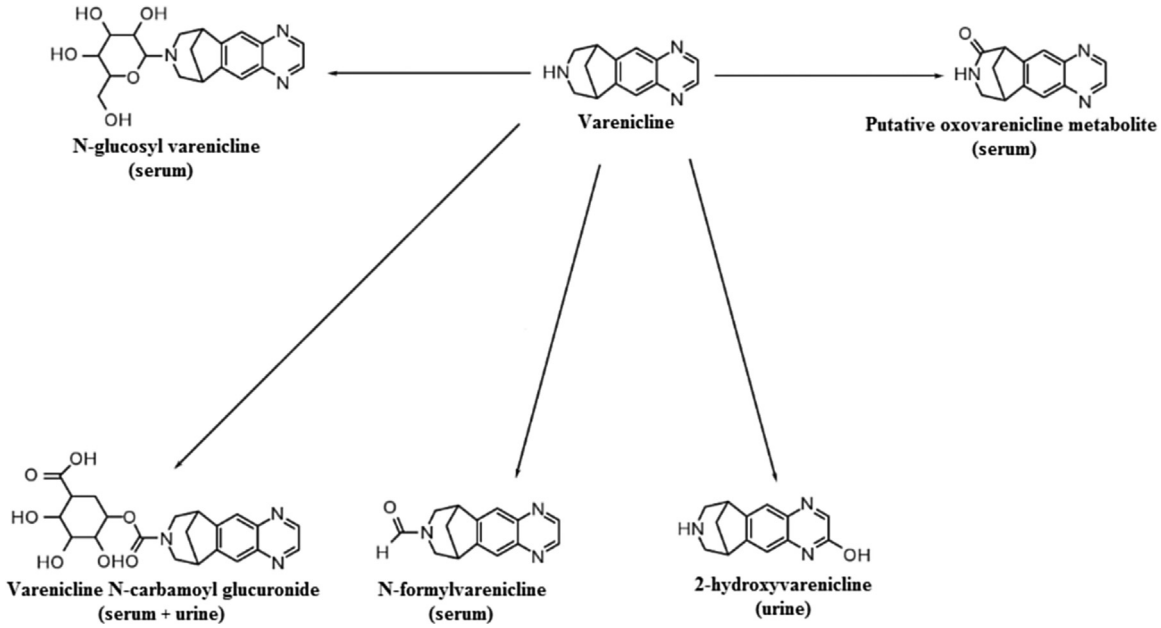


**Fig. 1.** Metabolism of varenicline in humans and excretion pathways of its metabolites [6].