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Attenuated Total Reflectance Fourier Transformation Infrared spectroscopy fingerprinted online monitoring of the kinetics of circulating Butyrylcholinesterase enzyme during metabolism of bambuterol

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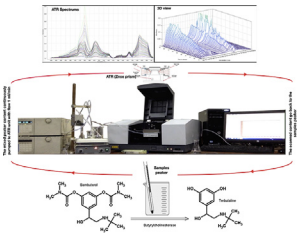
HIGHLIGHTS

- A system for measuring Butyrylcholinesterase enzyme kinetics established using Human serum, HPLC pump, ATR-FTIR unit.
- The system measures the product of bambuterol metabolism.
- A Validated method for determination of terbutaline was established.
- Characteristics and differences of the ATR-FTIR spectrums of BAM and TER were studied.
- Finally the reaction Kinetics (Km and Vmax) of BAM metabolism were studied.

GRAPHICAL ABSTRACT



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ABSTRACT

We have described a continuous flow ATR-FTIR method for measuring some of the Butyrylcholinesterase enzyme kinetics (K_m and V_{max}). This is done by developing a circulating system to be close as much as possible to the human circulation using human serum as a source of the enzyme with adjusted pH, isotonicity and temperature to give the maximum affinity of the enzyme towards its substrate (bambuterol).

The experiment was running continuously for 90 min to monitor the production of terbutaline from the zero time of its appearance with a measured spectrum in each minute using ZnSe prism. The method was selective and successful for determination of V_{max} to be 8.16×10^{-8} mol/min/ml and K_m to be 2.28×10^{-5} mol, showing high affinity of the enzyme towards its prodrug substrate Bambuterol.

This study critically probes the quantitative ability of the ATR-FTIR method for terbutaline, which was validated according to ICH guidelines showing high accuracy 100.39% and high selectivity towards the produced terbutaline, as the produced spectrums considered as fingerprint of each compound.

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1. Introduction

Butyrylcholinesterase (pseudocholinesterase) (BCHE) is a very important enzyme which is synthesized in liver under the control of BCHE genes [1] then distributed to numerous body sites, including plasma [2,3] this means that, it can be phenotyped via a single blood test [1] without the need for probe drug administration (as is done with cytochrome P450 enzymes which is found in hepatocytes, small intestine, lungs, kidneys and brain cells [4,5]).

Deficiency in this enzyme is probably the example most widely recognized clinically of an inherited defect in drug metabolism that affects outcomes with drugs. The clinical relevance is primarily in anesthesia either in normal surgeries or caesarian in which genetic BCHE deficiency or inhibition with drugs like Bambuterol (BAM) [6], predisposes to marked prolongation of paralysis or death with the two anesthetic neuromuscular blockers, suxamethonium and mivacurium [7].

BCHE has a very low investigation data because only few therapeutic compounds other than the neuromuscular blockers (suxamethonium and mivacurium) have some metabolism by BChE [8], including bambuterol [9] and cocaine [10].

Based on our knowledge, the only reported method for determination of BCHE activity was sophisticated and using cocaine as substrate [11] which is a narcotic drug that is not usually available and may cause addiction so Bambuterol (BAM) was chosen in our proposed method for the study of the activity of BCHE due to its availability in the market also BAM by itself has no bronchodilating effect, but it is metabolized in vivo by plasma Butyrylcholinesterase (BChE) into the pharmacologically active compound, Terbutaline (TER). So that the need of a method for determination of BCHE activity is of an urgent clinical importance.

Bambuterol is the inactive bis-*N,N*-dimethyl carbamate prodrug of the β_2-adrenoceptor agonist Terbutaline (TER) [12]. The active moiety is formed through hydrolytic and/or oxidative pathway [9]. The hydrolytic reactions are catalyzed mainly in plasma and the oxidative reactions most likely by cytochrome P450 (CYP) enzymes in liver or small intestine [13]. BAM is also an inhibitor of BCHE, with enzyme activity suppressed by 90% at 2 h after a 20-mg single dose [14] and 50% at 24 h on a steady-state regimen of 20 mg daily. Thus, BAM inhibits its own metabolism leading to prolongation of its bronchodilating activity leading to the advantage of being used once daily [15].

Administration of 20 or 30 mg BAM, two hours before anesthesia with suxamethonium or mivacurium in phenotypically normal patients resulted in prolongation of the neuromuscular blockade by 3- to 4-fold and may cause patient death [7].

Methods for online measuring or monitoring of drugs changes using different analytical techniques in biological fluids (Bioanalytical techniques) are becoming increasingly important for the study of bioavailability, bioequivalence studies, quantitative evaluation of drugs and their metabolites, diseases diagnosis, clinical pharmacokinetics and new drugs development [16–19].

Fourier Transform Infrared (FTIR) is a well-established technique in literature [20,21], has emerged as a very important analytical tool for qualitative and/or quantitative analysis of biological factors in a very complex biological samples such as (lung [22], prostate [23] and cervical [24]) cancer biomarkers, protein analysis [25], forensic science investigations [26] and petroleum components analysis [27,28] due to its non-destructive, high selectivity, high sensitivity towards Nanograms [29–31] and Picograms [32–35] of the under investigation substances in complementary with other techniques.

The infrared light absorption due to excitation from the ground vibration energy state to the higher vibrational states, gives characteristic information on the molecular structure and molecular interactions, that considered as fingerprint of any under investigation compounds [36].

Attenuated Total Reflectance (ATR) combined with FTIR is based on the fact that IR light is totally reflected at the boundary between two media. Therefore, ATR-FTIR is an excellent technique to study the interfacial region even in presence of water that considered as a strong absorbing solvent, so this technique can be used for biological and environmental applications [37].

When IR under investigation active moieties are found in the interfacial region, it creates a significant absorption that allows for identification of specific function groups and specific moieties within individual molecules [38].

ATR-FTIR technique can easily provide quantitative determination of concentration of certain substances within biological samples. Using horizontal ZnSe ATR prism, the IR beam undergoes multiple reflections. These multiple reflections considered as the summation of discrete reflections increasing the ability of rapid and quantitative determinations of concentrations of analyte using Beer's lambert law given in the following equation: -

$$A = \mathscr{E}\, l\, C = \mathscr{E}\, n\, d_e C$$

where $\mathscr{E}$ is the molar absorptivity, C is the unknown concentrations, n is the number of active internal reflections at the interface and d_e is the effective penetration depth [39].

Most methods for determination of enzymes activity, either depend on determination of the decrease of the amount of substrate or increase of the amount of the produced metabolite [40].

The current proposed method depends on monitoring of the enzyme activity by quantification of the amount of the increasing metabolite Terbutaline (TER) using ZnSe ATR prism implemented within a continuous circulation that runs continuously for 90 min.

This circulation was assembled to match the human circulation as much as possible consisting of BAM as a substrate of BCHE enzyme, Human serum as the source of BCHE enzyme dissolved in a proper buffered saline at normal human body temperature, HPLC peristaltic pump that simulate the human heart to pump all the contents with constant flow all connected to ATR-FTIR for detection of the increase of the produced metabolite TER to get a representative data for determination of the BCHE activity parameters K_m and V_{max} [41]. Michaelis Menten constant (K_m) shows the concentration of the substrate when the reaction velocity is equal to one half of the maximal velocity for the reaction. It can also be thought of as a measure of how well a substrate complexes with a given enzyme, otherwise known as its binding affinity. V_{max} represents the maximum velocity achieved by the system, at maximum (saturating) substrate concentrations [42].

This study critically probes the implementation of online ATR-FTIR technique in such reaction system which could open up several benefits such as, the ability for qualitative and quantitative determination of the metabolites, the reduced efforts and errors of offline sampling, the ability to monitor and analyze the produced spectrums continuously (online) and analyze samples directly in any time without any intervention to pause the reaction at any point of time.

2. Experimental procedure

2.1. Materials and reagents

- Bambuterol HCl (Purity 99%) was kindly supplied from AstraZeneca, Egypt.
- Terbutaline Sulphate (Purity 98%) was purchased from Sigma Aldrich.

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