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# Detection and quantitation methods for β-blockers

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**Abstract:** Beta-blockers, also known as β-blockers, are a class of medications commonly prescribed to treat arrhythmias (Drugs such as Bisoprolol, metoprolol succinate), prevent secondary heart attacks, and sometimes manage hypertension. These drugs inhibit the activity of endogenous catecholamines—namely epinephrine and norepinephrine—within the sympathetic nervous system by targeting adrenergic beta-receptors. While some beta-blockers non-competitively block all beta-adrenergic receptors, others do so in a competitive manner. It is targeted at the beta receptor on the cell wall, selectively binds to the beta adrenergic receptor, antagonizes the excitatory effect of neurotransmitters and catecholamines on the beta receptor, and is mainly used in the treatment of hypertension, coronary heart disease, heart failure and other diseases, which can cause adverse reactions. Moreover, It may cause bradycardia, second degree type II and above atrioventricular block, bronchial asthma, and hypotension. Central nervous system adverse reactions, multiple dreams, hallucinations, insomnia, fatigue, vertigo and depression and other symptoms, especially high-fat soluble β-blockers, easy to cross the blood-brain barrier to cause adverse reactions, such as propranolol. The commonly used β-blockers in clinic include metoprolol, atenolol and ecolol. In the United States Food and Drug Administration (FDA) classification of drugs according to the degree of harm to pregnant women, most drugs are classified as class C or D.

**Keywords:** β-Blockers, Anti-Doping, Electrochemical Methods, Liquid Chromatography.

## 1. Introduction

Beta-adrenergic antagonists, commonly referred to as beta-blockers or β-blockers, represent a class of pharmaceutical agents frequently prescribed for the management of arrhythmias, the prophylaxis of recurrent cardiac events, and, in some instances, the control of hypertension. These pharmacological agents exert their effects by impeding the functioning of endogenous catecholamines, specifically epinephrine and norepinephrine, within the sympathetic nervous system. This inhibition is achieved through the selective targeting of adrenergic beta-receptors. It is noteworthy that certain beta-blockers competitively block beta-adrenergic receptors, whereas others do so in a non-competitive manner.

The categorization of these medications into two distinct generations is predicated upon their receptor specificity. First-generation beta-blockers, exemplified by propranolol, nadolol, and sotalol, exhibit non-selective antagonism towards both beta1 and beta2 adrenoceptors. In stark contrast, second-generation beta-blockers, including atenolol, bisoprolol, and metoprolol, manifest a predilection for beta1-adrenergic receptors, rendering them particularly efficacious in the management of heart failure.

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Atenolol, carvedilol, and metoprolol are commonly listed during the 30 most commonly prescribed medications in the USA. These drugs offer several therapeutic benefits: they are effective in treating arrhythmias from various etiologies, lowering blood pressure in hypertensive patients, and reducing heart rate. Additionally, they are useful in managing typical angina pectoris and improving cardiac function. Beyond these applications, they can also be used to lower basal metabolic rate and control symptoms like agitation and tachycardia.

While they are invaluable in various medical contexts, beta-blockers can be toxic at high concentrations. An overdose can lead to a critically low, or even life-threatening, decrease in heart rate. Furthermore, these drugs are sometimes used in specialized sports competitions that demand precise motion control and steadiness.

Beta-blockers are considered first-line antihypertensive treatments in medical practice, effective in lowering blood pressure, although their efficacy is relatively moderate. They are best suited for managing mild to moderate hypertension. Antihypertensive medications are designed to control and manage elevated blood pressure, which often requires ongoing adjustments to both cardiac output and peripheral resistance.

The spectrum of antihypertensive medications can be divided into six primary classes. First are the Calcium Antagonists, which include medications like amlodipine, isradipine, and nifedipine. These drugs function by inhibiting the flow of calcium ions into the cells of the myocardium and vascular smooth muscle, thus lowering intracellular calcium levels and inducing functional changes in cardiovascular tissues. Second are the Angiotensin-Converting Enzyme (ACE) Inhibitors such as perindopril, benazepril, and captopril. These medications not only reduce blood pressure but also inhibit ventricular remodeling and decrease mortality rates in patients with heart failure. Third are the Angiotensin II Receptor Antagonists, represented by drugs like losartan and valsartan. These drugs are effective in lowering blood pressure and are usually well-tolerated, with coughing rarely reported as a side effect. Fourth are the Diuretics, including drugs like hydrochlorothiazide, furosemide, and tolazamide, which vary in their mechanisms and sites of action within the body. Fifth are the Beta-Receptor Blockers, primarily metoprolol and sotalol, which provide cardiovascular protection and are effective against hypertension and myocardial ischemia. Finally, there are the Receptor Blockers such as terazosin and phentolamine. These drugs block vascular receptors and dilate blood vessels, effectively reducing blood pressure. They are characterized by their potent effects and long-lasting action.

Beta-blockers function by impeding the binding of norepinephrine and epinephrine to beta-receptors located on nerve cells. Consequently, this inhibition leads to a reduction in heart rate and blood pressure. Additionally, it can induce airway constriction through the stimulation of muscle contraction surrounding the air passages. These pharmaceutical agents find application in the management of a diverse array of medical conditions, encompassing abnormal heart rhythms, hypertension, heart failure, angina, tremors, and pheochromocytoma. Furthermore, they demonstrate efficacy in migraine prevention and the mitigation of the risk of subsequent heart attacks and associated mortality. Beta-blockers also serve in the treatment of hyperthyroidism, akathisia, and anxiety disorders, with specific types exhibiting the capacity to lower intraocular pressure in individuals afflicted by glaucoma.

Nevertheless, apprehensions pertaining to their safety, tolerability, and potential adverse effects have constrained their broader clinical utilization. Frequently encountered side effects encompass gastrointestinal disturbances such as diarrhea, stomach cramps, muscle cramps, and nausea. Dermatological manifestations, including skin rashes, along with ocular issues leading to blurred vision, and generalized fatigue, are also reported. Central nervous system-related effects may encompass headaches, depressive symptoms, dizziness, nightmares, and hallucinations. Additionally, it is essential to acknowledge that beta-blockers can influence blood glucose levels and may obscure the clinical manifestations of hypoglycemia, especially in diabetic patients.

Various analytical techniques are employed for the study and measurement of these medications, including thin-layer chromatography, fluorescence, phosphorescence, capillary electrophoresis, gas chromatography, and mass spectrometry, to name a few. The most representative analytical methods in this regard are Liquid Chromatography and its related techniques, along with Electrochemical methods.

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## 2. Liquid Chromatography and Related Methods

Liquid chromatography (LC) is a technique of seperation that exploits variances in the distribution ratio of constituents within a mixture, distinguishing them between a liquid mobile phase and a stationary phase, which can be either solid or liquid in nature. Contemporary liquid chromatographic instruments encompass a comprehensive array of components, including a high-pressure infusion pump, a sampling system, temperature control, a chromatographic column, a detector, and a signal recording system. These advanced LC systems exhibit superior efficiency, velocity, and sensitivity when compared to classical LC apparatus. Nevertheless, it is important to note that LC is not well-suited for the separation of volatile compounds, as gas chromatography is better suited for such analyses. For analytical LC methods to yield dependable results, it is imperative that the vapor pressures of the analytes remain below that of the mobile phase.

LC methods are applied to tablets, capsules, ophthalmic and oral liquids, biological samples like urine, plasma, serum, aqueous humor, tissues, and environmental samples like water.

Sample preparation is very important in LC analysis. Matrix components can interfere with detection or analytes may be below detection limits. Thus, cleaning or preconcentration are performed before analysis to remove matrix components and improve sensitivity.

Simple protein precipitation with acetonitrile or methanol is used to prep plasma and serum samples.

In LC systems, the mobile phase from a reservoir is pumped at high pressure into a sampler where the sample enters the flow. The mobile phase carries the sample into the chromatographic column (stationary phase). Due to differing distribution coefficients between phases, components separate based on differences in mobility. Separated analytes which flow from the column into a detector which converts sample concentration into an electrical signal sent to a recorder. Key LC system components are the injection, infusion, separation, detection, and data processing systems.

Itohda and colleagues [1] conducted an investigation employing Solid-Phase Extraction (SPE) to determine the concentration of celiprolol in human plasma. The quantification of this analyte was accomplished through the utilization of High-Performance Liquid Chromatography (HPLC) in conjunction with a fluorescence detector (FLD). As an internal standard (IS), acebutolol was employed, as depicted in Table 1.

**Table 1.** provides an overview of the pharmacokinetic parameters associated with celiprolol subsequent to the administration of a single 200 mg dose, administered either with water or apple juice (n = 5).

| Parameter | Water | | Apple juice | | Apple juice/water |
|---|---|---|---|---|---|
| | Geometric mean | CV (%) | Geometric mean | CV (%) | Ratio (95% CI) |
| tmax (h) | 4 | | 8 | | |
| Cmax (ng/ml) | 397.0 | 50.5 | 23.0 | 39.3 | 0.058 |
| AUC24 (ng h/ml) | 1829 | 40.1 | 284 | 35.0 | 0.16 |
| t1/2 (h)a | 5.3 | 22.5 | 9.0 | 15.7 | 1.7 |

Here, we provide definitions and explanations for the abbreviations and terms used in our analysis:

1. CV (Coefficient of Variance): A statistical measure representing the relative variability of data points within a dataset.

2. CI (Confidence Interval): A range of values that is likely to include the true population parameter with a certain level of confidence.

3. tmax (Time to Reach Cmax): The median time it takes for a substance to reach its peak plasma concentration (Cmax) within a specified range.

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4. Cmax (Peak Plasma Concentration): The highest observed concentration of a substance in the bloodstream after administration.

5. AUC24 (Area Under the Plasma Concentration–Time Curve from 0 to 24 h): A pharmacokinetic parameter that quantifies the total exposure to a substance within a 24-hour period.

6. t1/2 (Half-Life): The time it takes for half of the substance to be eliminated from the body or reach half of its initial concentration.

Note: In this specific study, "a n = 4" signifies that the analysis was conducted with a sample size of four individuals. It's important to highlight that, in one volunteer, the t1/2 calculation was not feasible due to a significant delay in the time to reach Cmax (tmax), which was recorded as 12 hours.

These definitions and explanations provide clarity on the key pharmacokinetic parameters and statistical measures used in our research analysis.

Sai and colleagues [2] demonstrated a methodology employing High-Performance Liquid Chromatography combined with a linear ion trap mass spectrometer (HPLC-linear ion trap-MS) to quantify a panel of beta-agonists and beta-blockers present in animal-derived food products.

Ansari and Karimi [3] proposed a technique for measuring the concentration of sotalol. They employed a combination of molecularly imprinted Solid-Phase Extraction (SPE) and High-Performance Liquid Chromatography (HPLC), along with an ultraviolet-visible (UV-Vis) detector for analytical purposes.

**Table 2**. Parameters Involving Calibration Curves, Linearity Ranges, and Limits of Detection (LOD) and Quantification (LOQ) Values.

| Drug | Lincar range (μg mL-1) | Slope (a) | Sa | Interccpt (b) | Sb | Sxy | R2(n-6) | LOD (μgL-1) | LOQ (μgL-1) |
|---|---|---|---|---|---|---|---|---|---|
| Carvedilol | 0.5-40 | 9,674 | 54 | -5,517 | 1,068 | 1,903 | 0.9999 | 0.14 | 0.42 |
| Dexammethasone | 0.25-40 | 5,422 | 105 | 841 | 1,917 | 3,911 | 0.9981 | 0.09 | 0.26 |
| Ketoprofen | 0.2-40 | 11,075 | 133 | -753 | 2,440 | 4,977 | 0.9993 | 0.06 | 0.19 |
| Metamizole | 1.0-40 | 4,984 | 84 | -2,149 | 1,653 | 2,946 | 0.9989 | 0.35 | 1.05 |
| Metoprolol | 1.0-40 | 2,553 | 37 | -2,403 | 808 | 1,200 | 0.9994 | 0.39 | 1.18 |
| Paracetamol | 0.2-40 | 9,582 | 91 | 1,712 | 1,668 | 3,403 | 0.9995 | 0.07 | 0.20 |
| Prednisolone | 0.25-40 | 5,981 | 70 | -23 | 1,277 | 2,604 | 0.9993 | 0.08 | 0.24 |
| Propranolol | 0.5-40 | 28,752 | 437 | 778 | 8,643 | 15,400 | 0.9991 | 0.14 | 0.43 |
| Salicylic acid | 1.0-40 | 7,722 | 117 | -4,416 | 2,522 | 3,975 | 0.9993 | 0.34 | 1.02 |
| Sotalol | 0.25-40 | 5,696 | 85 | -89 | 1,675 | 2,984 | 0.9991 | 0.10 | 0.29 |

Boonjob and colleagues [4] (see Figure 1) conducted an evaluation of several commercially accessible sorbents, which encompassed Oasis MCX, Bond Elute Plexa, Bond Elute Plexa PCX, Oasis MAX,Oasis HLB, Bond Elute Plexa PAX and SupelMIP. The objective of their study was to assess the suitability of these sorbents for the purpose of Solid-Phase Extraction (SPE) applied to atenolol, pindolol, acebutolol, metoprolol, labetalol, and propranolol.

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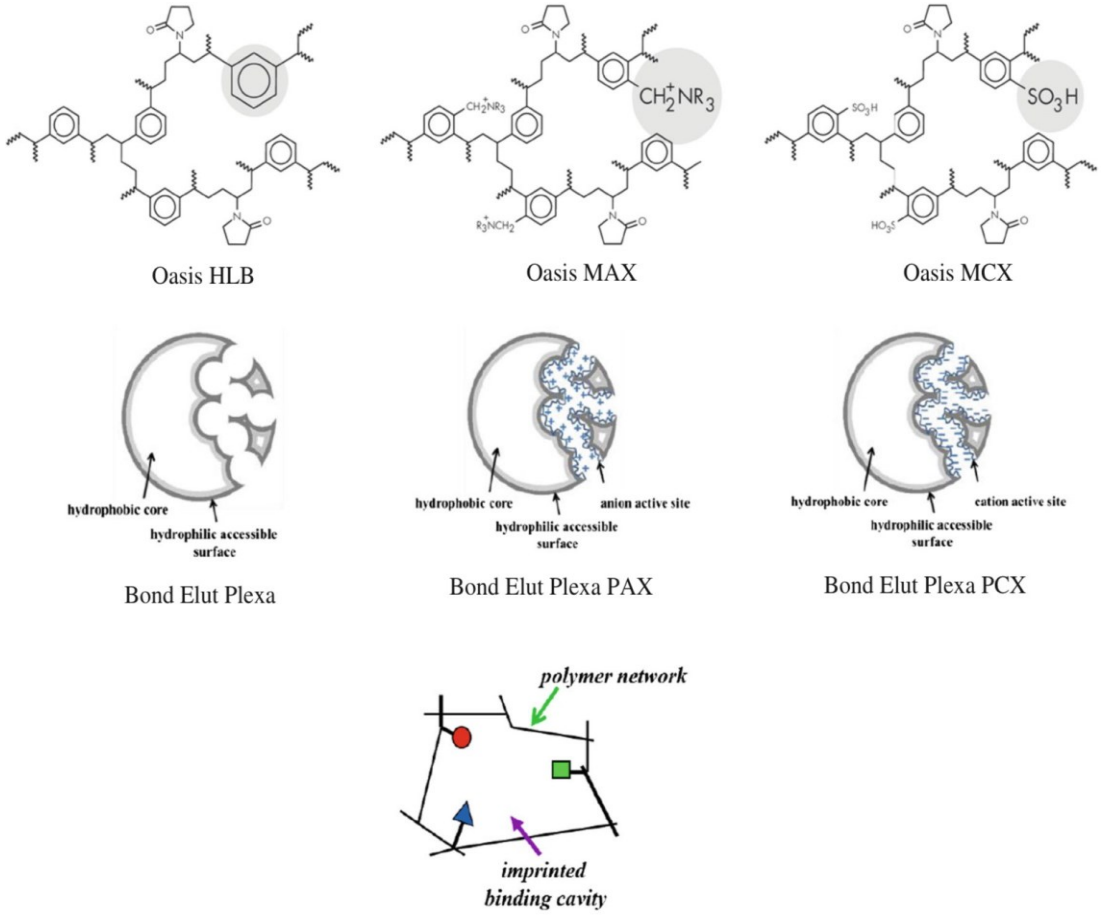


**Figure 1**. Structures of Oasis and Bond Elut Plexa Sorbent Families.

In addition to conventional manual techniques, the past decade has witnessed the emergence of automated Solid-Phase Extraction coupled with High-Performance Liquid Chromatography (SPE-HPLC) methods. Satinsky and colleagues [5] introduced an online approach that integrates microextraction by packed sorbents (MEPS) with HPLC-FLD for the quantification of metoprolol, labetalol, and propranolol in urine specimens.

HPLC separations have predominantly been executed in the reverse-phase (RP) mode. Xu and associates devised an HPLC methodology incorporating UV detection for the quantification of metoprolol and its two metabolites in urine. Similarly, Jouyban and collaborators presented a straightforward, expeditious, and highly sensitive technique that combines dispersive liquid-liquid microextraction (DLLME) for sample preparation with HPLC-UV separation for the determination of metoprolol, propranolol, carvedilol, diltiazem, and verapamil in plasma samples.

In adsorption chromatography, the stationary phase is the adsorbent material, and the separation process takes place on its surface, rather than within the interior of the stationary phase. Unlike in gas chromatography, molecules of the mobile phase (i.e., the solvent) are also adsorbed onto the adsorbent surface. On this surface, the sample molecules vie with mobile phase molecules for adsorption sites, making the choice of mobile phase crucial for separation efficiency. Generally, gradient elution techniques can enhance this efficiency. In polymer analysis, adsorption chromatography is commonly used for separating additives like azo dyes, antioxidants, and surfactants.

In conclusion, liquid chromatography systems are characterized by their high efficiency and rapid sensitivity. They find applications across various fields including biochemistry, biomedicine, and

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environmental chemistry. Depending on the nature of the stationary phase—whether it is liquid or solid—these systems are categorized as either Liquid-Liquid Chromatography (LLC) or Liquid-Solid Chromatography (LSC).

## 3. Electrochemical Methods

Electrochemical methodologies stand as formidable tools for assessing the chemical composition and characteristics of substances by monitoring alterations in current or voltage. These methodologies encompass diverse techniques, including electrochemical analysis, electrochemical synthesis, and electrochemical determination. Notably, one of the primary merits of electrochemical methodologies lies in their capacity to sensitively and selectively ascertain beta-blockers while maintaining cost-effectiveness, obviating the necessity for distinct extraction or purification steps. Furthermore, these methods exhibit reduced susceptibility to interference from light absorption and fluorescence in comparison to spectroscopic approaches and can be readily adapted with nanomaterials.

Abou Al Alamein and colleagues devised an analytical approach employing chemometrics-assisted voltammetry for the quantification of timolol maleate. This method utilized a carbon paste electrode modified with iron (III) oxide nanoparticles. Their investigation harmonized electrochemical techniques with chemometric tools, resulting in the development of a robust and highly sensitive analytical method [6].

In the domain of bioanalysis, Mohammed and collaborators (refer to Table 3) introduced a technique employing nafion/carboxylated multi-walled carbon nanotube nanocomposites for the analysis of minute quantities of timolol maleate in urine samples. This novel approach exhibited swift response times, abbreviated analytical duration, heightened sensitivity, and exceptional selectivity, rendering it particularly well-suited for the quantification of timolol in diverse matrices such as eye drops, urine, and water specimens [7].

**Table 3**. Comparison between the modified method and other methods.

| Technique | Linearity (mol L-1) | LOD (mol mL-1) |
|---|---|---|
| SWV-ME | | 2.50×10-5 |
| RP-HPLC | | 9.25×10-4 |
| RP-HPLC | | 5.75×10-3 |
| MEC | 1.6×10-2-2.0×10-2 | 1.20×10-1 |
| Spec | - | 3.00×10-2 |
| HPLC | - | 4.60×10-6 |
| CL | 2.3×10-3-1.2×10-5 | 1.76×10-5 |
| DPACSV-HMDE | 1.0×10-7-1.5×10-6 | 1.26×10-6 |
| HPLC-UV | 1.5×10-7-6.3×10-6 | 2.53×10-6 |
| CZE | 6.3×10-5-3.16×10-4 | 4.3×10-2 |
| DPASV-MGCE | 1.0×10-9-5.0×10-7 | 7.10×10-7 |

Various analytical techniques and methodologies are employed in scientific research, each with its distinct abbreviation:with a Mercury Electrode (SWV-ME.) Micellar Electrokinetic Chromatography (MEC); Reverse Phase High-Performance Liquid Chromatography ((RP-HPLC); Spectrophotometry (Spec); High-Performance Liquid Chromatography (HPLC); Chemiluminescence (CL); Differential Pulse Adsorptive Cathodic Stripping Voltammetry with a Hanging Mercury Drop Electrode (DPACSV-HMDE); Capillary Zone Electrophoresis (CZE); ifferential Pulse Adsorptive Stripping Voltammetry with a Modified Glassy Carbon Electrode (DPASV-MGCED).

These abbreviations are employed throughout the research to denote specific analytical methods and techniques, each contributing to the comprehensive analysis of various compounds and substances.

Molecularly imprinted polymers have emerged as important candidates for selective sorbent materials. Nezhadali et al. (Table 4) developed an electrochemical sensor based on a new MIP for ultra-trace level detection of metoprolol in serum samples. Their approach was simple, fast, and economically efficient [8].

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**Table 4.** Results of MTP determination in samples.

| Sample | MTP added (μM) | Average of MTP found (μM) ±RSD | Recovery (%) |
|---|---|---|---|
| Serum sample | 0 | Not detected | - |
| | 20 | 19.76 ± 0.02 | 98.80 |
| | 40 | 39.42 ± 0.02 | 98.55 |
| Tablet(Alborzdarou Co. | 0 | 19.06 ± 0.03 | - |
| | 20 | 39.91 ±0.01 | 104.25 |
| | 40 | 58.82 ± 0.02 | 99.40 |
| Tablet(Poorsina Co. | 0 | 20.42 ± 0.02 | - |
| | 20 | 40.03 ±0.03 | 98.025 |
| | 40 | 59.33 ± 0.01 | 97.26 |
| Tablet(Hexal Co.) | 0 | 20.13 ± 0.02 | - |
| | 20 | 40.49±0.02 | 101.80 |
| | 40 | 61.99 ± 0.02 | 104.65 |

Enantioselective electrochemical sensors offer a rapid and cost-effective alternative for chiral discrimination of electroactive compounds. Chen et al., for example, designed a chiral sensor using multi-walled carbon nanotubes (MWCNTs) and ionic liquids (ILs) nanocomposite for enantiomeric recognition of propranolol. Likewise, Stoian et al. explored the enantiomeric interactions of propranolol on surfaces modified with L-cysteine and gold nanoparticles. Such developments pave the way for future chiral electrochemical sensors that are quick, cost-effective, and reliable.

## 4. Conclusion

In summary, this essay has reviewed the impacts of both liquid chromatography and electrochemical methods on the analysis of beta-blockers. Liquid chromatography systems, characterized by high efficiency and rapid sensitivity, find applications in biochemistry, biomedicine, and environmental chemistry. These systems are further classified into Liquid-Liquid Chromatography (LLC) or Liquid-Solid Chromatography (LSC) based on the nature of the stationary phase.

Electrochemical methods are increasingly favored for their multitude of advantages, including high sensitivity, cost-effectiveness, and low sample volume requirements. Advanced nanomaterials offer the potential for ultra-sensitive electrode surfaces, especially for biological samples. While this study did not explore the simultaneous detection of multiple beta-blockers with a single electrochemical sensor, future research could employ virtual or multiple template molecules to realize this goal.

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