# Oxidative and metabolic stability of drugs: assessment by a novel Electroactive Pharmaceutical Screening System

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A major focus observed in recent years in drug development is to reduce the number of compound failures at a late stage. Such misfortune is frequently the result of unfavourable compound properties, like hepatic toxicity, metabolic instability or slight potency. Rational drug discovery begins with the knowledge of stability and reactivity of libraries and early prediction of properties (e.g. drug-likeness, ADME, toxicity), that influence the success of a drug candidate at the end of development. To address this challenge, Gatlik® Company has introduced the **Electroactive Pharmaceutical Screening (EPS) System** to allow more informed and accurate decisions about synthesis and lead selection in the early stages.

Chemical oxidative stability is a significant concern at all stages of drug development. A tendency of a chemical substance to undergo oxidation can be determined from its electrochemical properties[1]. An extensive literature survey shows that there is a strong relation between a compound's tendency to undergo oxidative degradation and its oxidation potential. The Gatlik® EPS System (Fig. 1) allows to perform parallel oxidation reactions of screened compounds and to determine their oxidation potential via a multiple voltammetric analysis. The comparison of oxidation data between compounds and standards of known oxidative properties is therefore a very powerful approach to predict the oxidative and metabolic stability of drug library candidates. The unique design (96-well format) of the EPS Sensor-Plates makes it the only system capable of automated, routine oxidation potential determination, relevant to oxidative and metabolic stability.


Figure 1. Gatlik® EPS System with Sensor-Plate-96.2TV3 and controlling & processing software.

## TECHNOLOGY

The Gatlik® EPS System is novel, automated high-throughput screening equipment, suitable for assessing the oxidative, reductive and metabolic stability of new drug candidates at the early stage of lead optimization. The determination is based on intrinsic electrochemical parameters of tested compounds and does not require any biological material. The EPS System allows to electrochemically produce the first pass metabolites of drugs in reactions like dehydrogenation, N-, S-, C- oxidation and N-, O- dealkylation with the activation reflecting Cytochrome P450 redox processes[2]. The innovative aspect of this analytical and production device is the application of electronic sensors for stability investigation of new chemical entities. Therefore, a conventional 96-well plate has been replaced with a 96-sensor plate, where electronic sensors and transducers are located in each well of the plate. The EPS device does not require any enzymes or organic material and is not restricted to certain compound types. The system principle was extensively evaluated by various assays and types of compounds. This innovative technology shows promising results for a variety of applications beyond metabolic screens, e.g. in formulation development, antioxidant profiling or hepatotoxicity prediction.

In detail, the Gatlik® EPS System consists of:

- Sensor-Plate-96.2TV3
- Transformating & analysing unit
- Controlling & processing software

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EXPERIMENTAL

**Chemicals.** All reagents were of the highest grade commercially available. Phosphate buffer pH 7.4 with 0.09% KCl and DMSO (Fluka), helium from Carbagas were purchased from the sources indicated. All the compounds (paracetamol, ascorbic acid, captopril, clozapine, procainamide, chlorpromazine, astemizole, diclofenac and sulfadiazine) submitted for screening were purchased from Sigma, Fluka and Aldrich.

**Instrumentation.** The electrochemical production and analysis were performed with the Gatlik® EPS System in a multi reaction Sensor-Plate type 96.2.T-V3 (Gatlik Ltd). The voltammetric current–potential data and also cathodic and anodic peak potentials were automatically collected and analysed by EPS Software on a Hewlett-Packard computer.

**Solution Preparation**. All drug solutions (at pH 7.4) were prepared by automated pipetting (Tecan Genesis) of drug stock solutions in DMSO to previously prepared phosphate buffer saline deoxygenated with helium. The working solutions contained 0.2 mM sample, 1 mM phosphate buffer pH 7.4, 0.09% KCl, and 5% DMSO. All drugs were handled with extreme care, and according to GxP standards.

RESULTS

**Oxidative Stability.** Using EPS System, the oxidised forms of drugs were produced and simultaneously oxidation potentials (EPSox) were measured. In Fig. 2 the voltammograms for oxidatively stable compounds are represented as solid lines while those of unstable compounds are represented as dashed lines. The experimentally determined oxidation potentials were highest for sulfadiazine (0.82 V) and diclofenac (0.71 V)

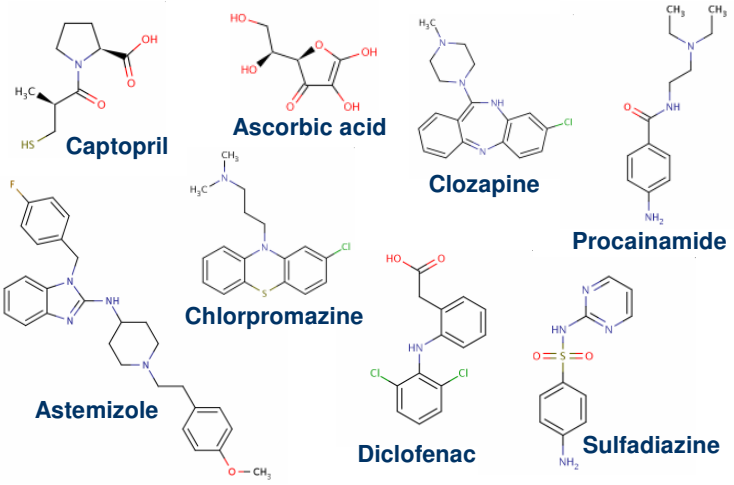


Figure 3. Chemical structures with "soft spots" in colour.

described as stable and middle for astemizole (0.67 V), chlorpromazine (0.48 V) and procainamide (0.455 V) described as moderately stable. The four samples: paracetamol (0.22 V), ascorbic acid (0.32 V), captopril (0.34 V) and clozapine (0.395 V), with the lowest measured oxidation potentials were described as unstable. Data determined by EPS System show a strong correlation between experimentally measured oxidation potentials and reported oxidative stability. The compounds shown in Fig. 3 were well characterized in terms of stability toward electron-transfer based oxidation[1]. From these and other studies, it was found that analysis of drug libraries using the Gatlik® EPS System allows a rapid and simple classification of compounds into stability categories under physiological-like conditions. Based on oxidation potential of drug candidates vs. Ag/AgCl reference electrode, the following simple classification rules may be applied: above 0.75 V stable, between

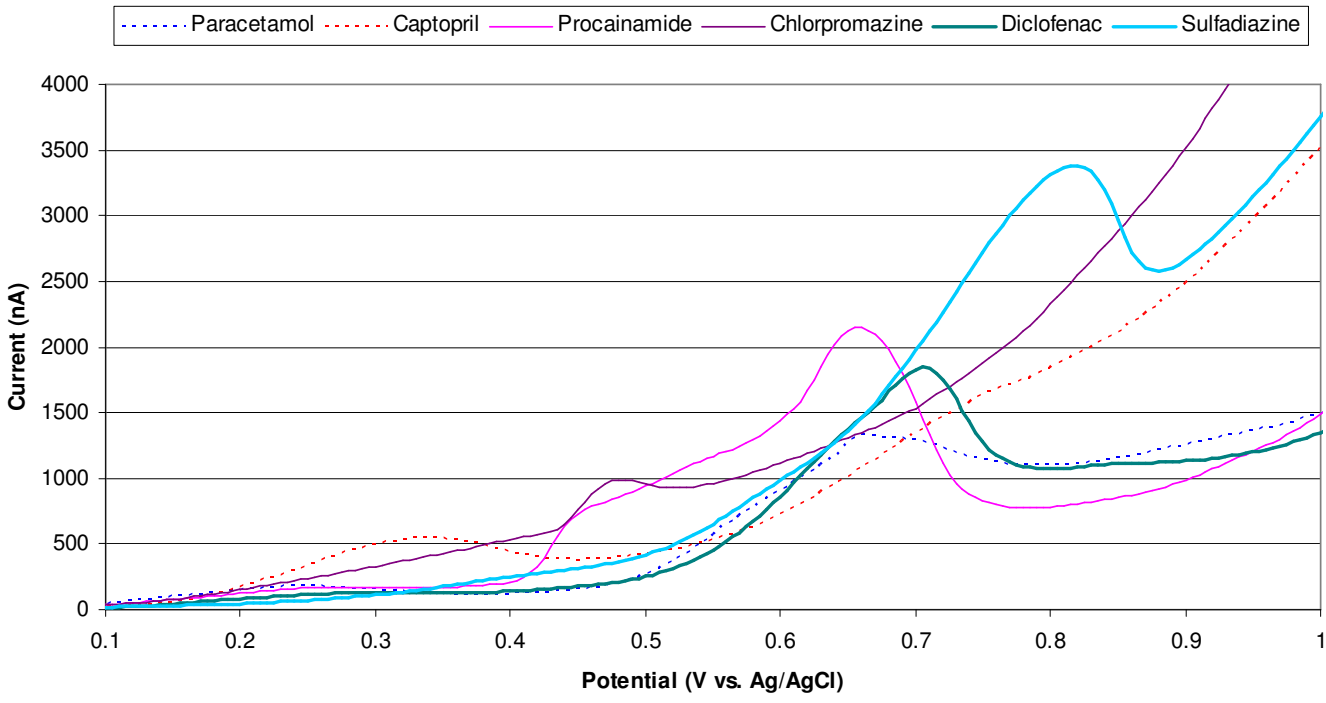


Figure 2. Voltammograms of the representative drugs in buffered solution at pH 7.4 recorded using the EPS System in high-throughput mode (scan rates 128 mV/s).

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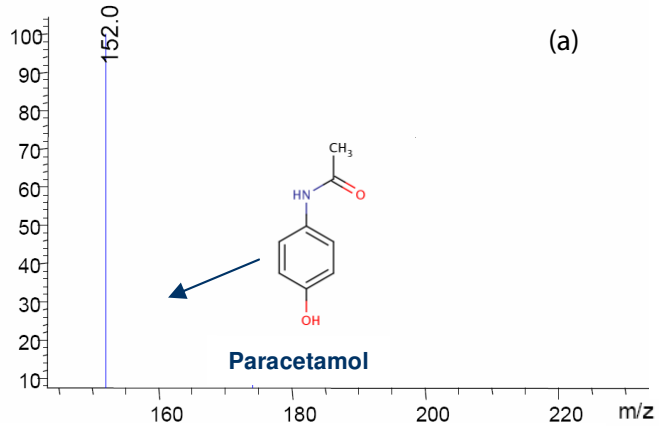
0.45 and 0.75 V moderately stable and below 0.45 V unstable. The screening capability of the EPS System allows more in-depth investigation of drug candidates through e.g. Quantitative Structure-Activity Relationship (QSAR) study, to provide more accurate knowledge of stability and reactivity. Application of these results in the early phase of lead design will contribute to better models, more precise predictions, correct selection, and consequently a higher success rate of rational drug discovery.

**Oxidation Products.** Generation and identification of metabolites and derivatives in the early stage of discovery is of great interest. The related substances may provide guidance for modelling, synthesis, candidate selection and assay development for metabolic or toxicity studies. EPS System coupled with mass spectrometry allows synthesis with subsequent qualitative analysis of product. The oxidative metabolism of paracetamol with the formation of reactive N-acetyl-p-benzo-quinone imine (NAPQI) is widely reported as a fundamental reason of its hepatic toxicity in humans[3]. Fig. 4 shows mass spectra obtained for paracetamol before (a) and after electrochemical oxidation (b). The mass-to-charge ratio (m/z) shift related to oxidative formation of NAPQI (m/z -2) through dehydrogenation reaction was detectable above EPSox 0.22 V. Similarly, the formation of p-benzo-quinone imine (PQI) (m/z -44) trough dehydrogenation and N-deacetylation reactions was measurable above the potential of 0.66 V. In Fig. 5 mass-to-charge ratio shifts corresponding to electrochemical formation of metabolites show that the nature of products is dependent on the applied potential during synthesis. This gives a suitable level of control over both yield and nature of electrochemical reactions by simple selection of the oxidation ranges. The control over product formation available in the EPS System helps in forced oxidative degradation studies and in predictive metabolic and toxicity investigations.

Figure 4. Mass spectra obtained for paracetamol.



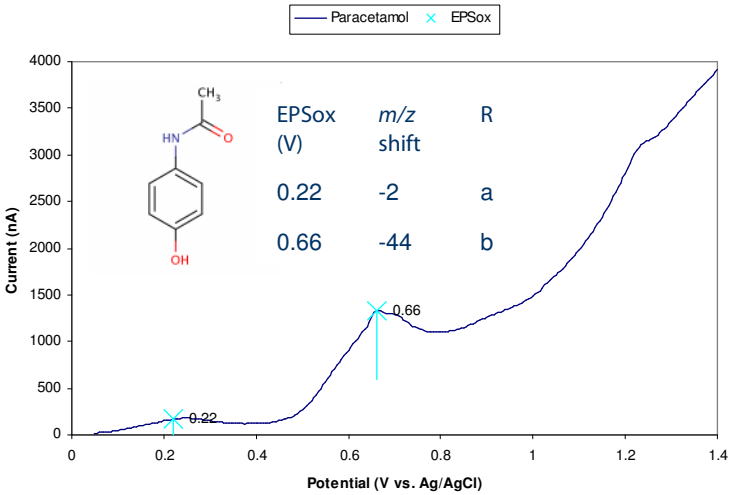




Figure 5. Voltammogram, m/z shifts and oxidation potentials (EPSox) of paracetamol used as substrate for (a) dehydrogenation and (b) N-deacetylation reactions (R).

## CONCLUSION

These results demonstrate that the EPS System may be used in high-throughput predictive stability studies and as a powerful tool for production of drug metabolites and derivatives in controlled electrochemical reactions. The parallel syntheses and stability determinations can be carried out under physiological-like conditions in a 96-well plate format. Since oxidative stability, bioactivity, metabolism, and toxicity frequently involve redox reactions, Gatlik® EPS System represents a powerful equipment that can be systematically applied to prediction and evaluation of drug properties. The presented novel technology due to its fast, cheap and precise determination, can be very helpful at the early drug discovery stage to allow well-informed and smart decisions about synthesis and lead selection for further successful development.

## ACKNOWLEDGEMENTS

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