Coupling biological detection to liquid chromatography as a solid tool for the separation, purification and online biological characterization of chemical compounds present in natural or synthetic mixtures

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The direct interaction of molecules, extracts and medicines with tissues or living organisms it is essential and unavoidable knowledge in the actual commercial therapy. Studies achieve during the preclinical and clinical stages of drug development and that are very useful to reveal the pharmacological profile of drugs.

We present a new system for the direct pre-characterization of hydrosoluble pharmacologically active substances with medium pressure liquid chromatography (MPLC) coupled to biological detection using perifused or perfused organs.

Coupling MPLC to perifused organs and organ perfusion cascade

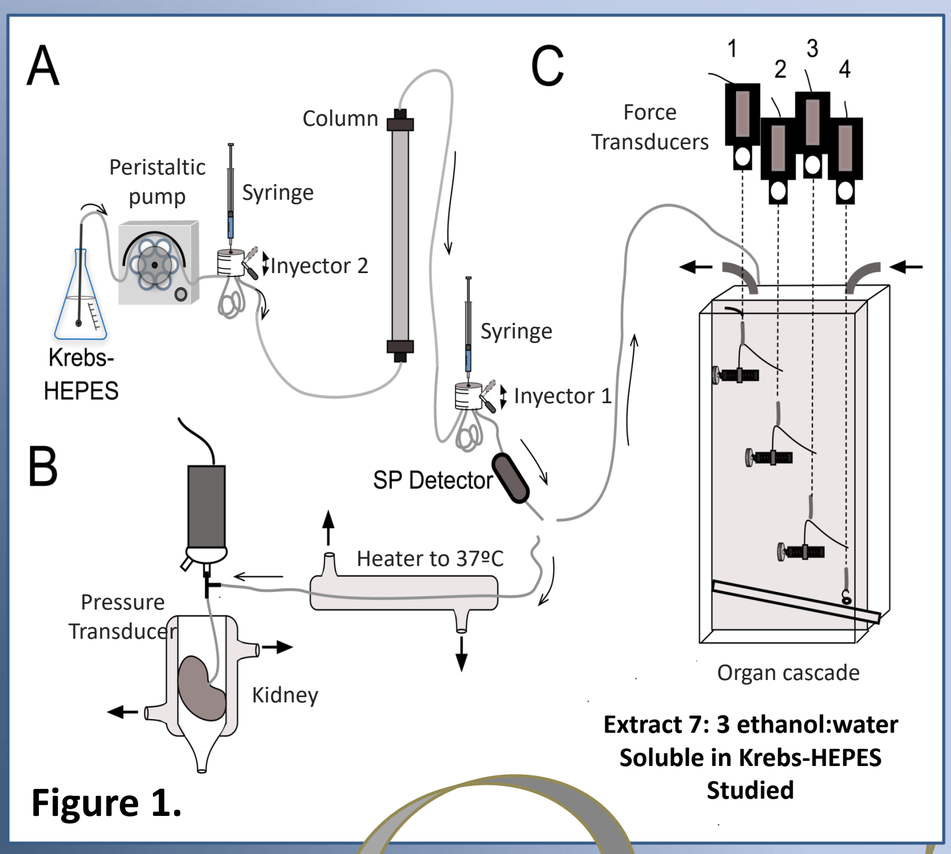
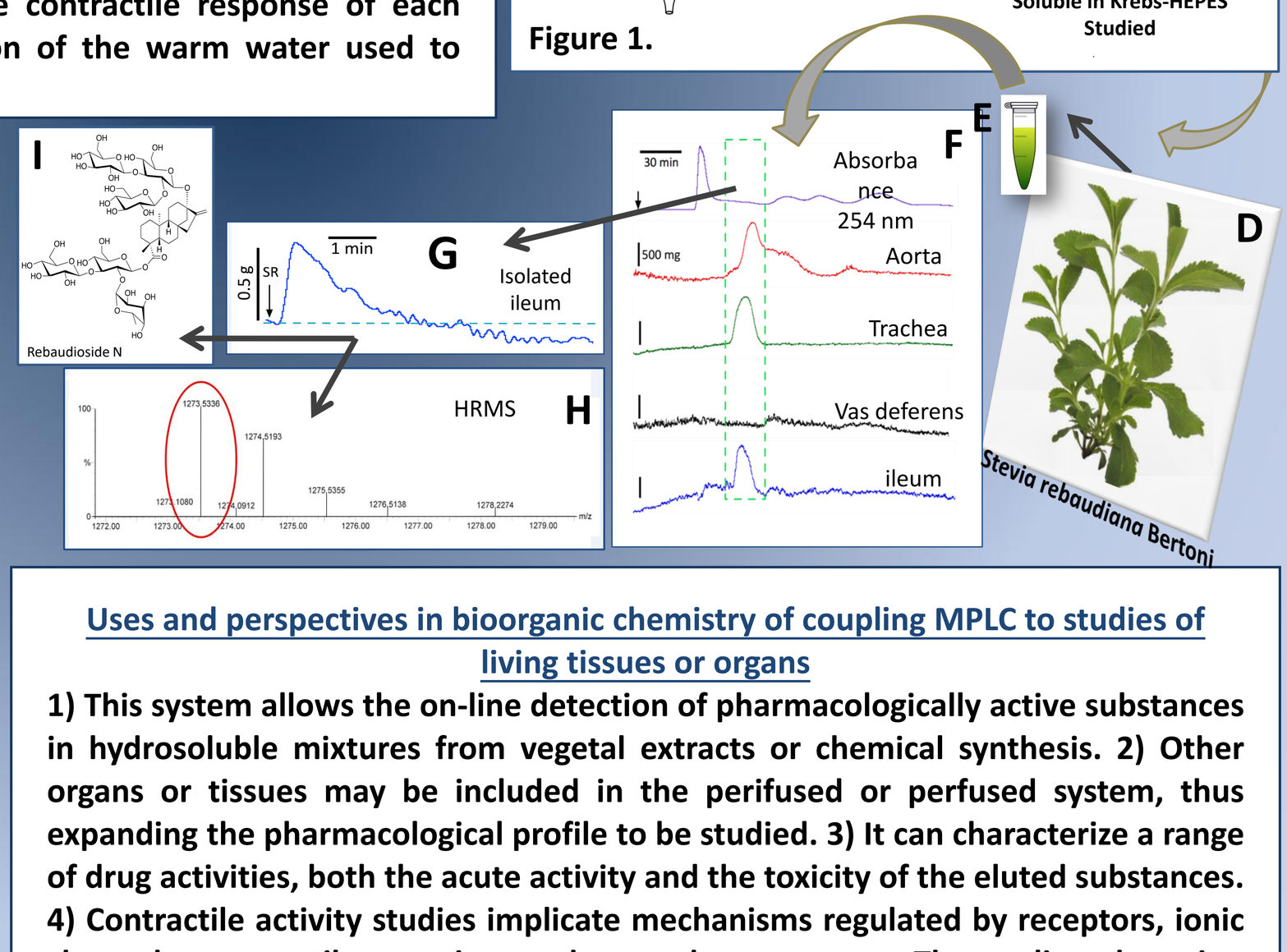


Figure 1. (A) A Krebs-HEPES solution, which acts as a mobile phase, is pumped through a standard roller pump to a 6-port injection valve (injector 2). Point of injection of the extracts that undergo chromatographic separation of molecular exclusion (Sephadex G-10) as it passes through the column. Another injection valve (injector 1) is placed after the column to calibrate the contractile responses of injected samples directly, without previous separation. The fluid that emanates from the column (gray arrows) passes through a spectrophotometric detector (SP Detector) and then, the fluid can be either conducted to a perfused kidney, the inward pressure of which is continuously monitored (B), or it can be diverted towards a series of superfused organs (aorta, trachea, vas deferens and ileum: cascade of organs) and the tension produced in each organ is monitored individually and continuously using force transducers(C). The tension values indicate the contractile response of each tissue. The black arrows indicate the circulation of the warm water used to maintain the preparations at 37 ° C.

Following this methodology an extract 7: 3 ethanol:water from the species *Stevia rebaudiana* Bertoni (D), a perennial herb native to Paraguay, was studied. The injection of samples of concentration 100 mg/ml (E) allowed to detect contractile activity in three of the studied tissues: aorta, trachea and ileum (F) in a fraction that did not show absorption peak in the detector at 254nm (nor at 210 and 280 nm). Using as a mobile phase water (A), with the same methodology and maintaining retention times, the fraction with equal retention time was analyzed in an organ bath showing potent contractile activity in ileum (G). The study of mass spectrometry in negative mode of the active fraction (H), allowed to identify the rebaudioside N (I) as the compound responsible for the contractile activity.



Uses and perspectives in bioorganic chemistry of coupling MPLC to studies of living tissues or organs

1) This system allows the on-line detection of pharmacologically active substances in hydrosoluble mixtures from vegetal extracts or chemical synthesis. 2) Other organs or tissues may be included in the perifused or perfused system, thus expanding the pharmacological profile to be studied. 3) It can characterize a range of drug activities, both the acute activity and the toxicity of the eluted substances. 4) Contractile activity studies implicate mechanisms regulated by receptors, ionic channels, contractile proteins, and second messengers. The on-line detection system will be useful for QSAR studies and the search for new drugs from a lead drug detected following the methodology described here.

This novel system of direct coupling of chromatography to biological detection has proved to can be a powerful tool to facilitate the pharmacological characterization of active compounds in mixtures derived from natural extracts or combinatorial chemistry libraries. This new method has several advantages over the classic way of testing natural products, avoiding tedious, expensive, and time consuming and animals slaughtered procedures.

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