Pharmaceutical and Biomedical Analysis Using Liquid Chromatography Coupled to Pre/Post Column Derivatization

Paraskevas D. Tzanavaras*
Laboratory of Analytical Chemistry, Department of Chemistry, Aristotelian University of Thessaloniki, GR-54124 Thessaloniki, Greece

Liquid chromatography (LC or HPLC) is the predominant technique in pharmaceutical and biomedical analysis. LC is fairly simple and the separation mechanism is rather straightforward and readily understandable compared to other more complicated separation techniques (e.g. capillary electrophoresis). A wide selection of specific and non-specific detectors and numerous analytical columns and stationary phases cover almost all possible applications. Furthermore, instrument manufacturers offer highly reliable instrumentation capable of automated operation on a 24-h basis.

When it comes to the analysis of complex biological material by LC methods, sample preparation is often the bottleneck of the whole analytical procedure. Matrix interferences should typically be removed and compounds of interest should be preconcentrated. An ideal sample preparation protocol should be simple, rapid, effective and if possible automated. One of the widely used sample pretreatment techniques in chemical analysis is derivatization. In principle, derivatization is the conversion of the analyte(s) in a form that is more suitable for analysis by the selected technique. The simplest way to derivatize a sample is by a chemical reaction using a reagent that is capable of reaction with a selected functional group of the analyte(s). For example a mixture of amino acids can be derivatized by using a reagent that is selective for primary amino moieties. Alternative and less common derivatization strategies can be based on reagent-less procedures such as photochemical and electrochemical conversions.

The main goals of derivatization as sample preparation technique can be summarized to the following highlights:

(i) Stabilization of sensitive samples (e.g. protection of thiolic analytes from oxidation to disulfides through derivatization of the –SH group).

(ii) Sensitivity enhancement (e.g. derivatization with fluorogenic reagents).

(iii) Alteration of the properties of the analytes in order to be compatible to the analytical technique (e.g. enhancement of the volatility by derivatization prior to gas chromatographic analysis)

(iv) Enhanced selectivity through group-specific reactions (e.g. primary versus secondary amino compounds)

In LC applications derivatization can be carried out in two basic modes, which are either pre-column or post-column (although there are several in-column procedures). In pre-column derivatization, LC separation is applied to the analytes derivatives. Derivatization can be carried out in a batch or automated mode. Automation is accomplished by using a suitable LC autosampler (many manufacturers provide autosamplers with mixing capabilities) or alternatively by coupling LC to automated flow techniques (FI, SI). Three main parameters have to be taken into account during the development of a method based on pre-column derivatization:

(i) The stability of the derivatives in the case of batch procedures

(ii) Potential interference and peak overlapping from the excess of the derivatization reagent or its degradation by-products.

On the other hand, post-column derivatization is an automated procedure per se. Analytes are separated in their native forms and on-line mixing with a suitable reagent is carried out downstream, after their elution from the chromatographic column. A typical flow setup of LC coupled to post-column derivatization is depicted in Figure 1. During development of a method based on-column derivatization the following aspects should be considered:

(i) The peaks are generally broader due to extra dispersion introduced by the post-column part of the setup.

(ii) The derivatization reagent must be selected taking into account the speed of reaction, the cost (it flows continuously) and the background signal. On the other hand, stability of the derivatives is not an issue due to the on-line character of the procedure.

(iii) Additional instrumentation is needed (propulsion pumps, reaction coils, on-line thermostats, back-pressure regulators etc) [1,2].

(iv) Post-column derivatization is particularly useful for the analysis of complicated samples. During development of such methods

![Figure 1: Schematic diagram of a representative setup of LC coupled to post column derivatization](image)

---

*Corresponding author: Paraskevas D Tzanavaras, Laboratory of Analytical Chemistry, Department of Chemistry, Aristotelian University of Thessaloniki, GR-54124 Thessaloniki, Greece, Tel: 0030 2310997721; E-mail: ptzana@chem.auth.gr

Received October 22, 2012; Accepted October 24, 2012; Published October 26, 2012


Copyright: © 2012 Tzanavaras PD, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
separation is the critical step that dictates the accuracy and efficiency of the procedure. If this is achieved, post-column reaction of the analytes is performed in “isolation” avoiding this way matrix effects and competition from endogenous sample compounds.

In the scientific literature there numerous interesting articles where the interested reader can find valuable information on theoretical and practical aspects of analytical derivatization coupled to liquid chromatography [3-17]. From my personal research experience I have concluded that post-column derivatization coupled to LC is my first choice for the analysis of biological material, where the sample matrix is more or less unknown and may vary (Figure 2) [18]. On the other hand, pre-column methods and especially automated ones can be readily applicable simple and controlled matrices e.g. to the quality control of pharmaceutical formulations.

References

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:
• User friendly/feasible website-translation of your paper to 50 world’s leading languages
• Audio Version of published paper
• Digital articles to share and explore

Special features:
• 200 Open Access Journals
• 15,000 editorial team
• 21 days rapid review process
• Quality and quick editorial, review and publication processing
• Indexing at PubMed (partial), Scopus, DOAJ, EBSCO, Index Copernicus and Google Scholar etc
• Sharing Option: Social Networking Enabled
• Authors, Reviewers and Editors rewarded with online Scientific Credits
• Better discount for your subsequent articles

Submit your manuscript at www.omicsonline.org/submission