Flow-injection analysis for multi-component determinations of drugs based on chemometric approaches

Javier Saurina

This article discusses the possibilities of flow-injection analysis (FIA) and related flow techniques in combination with chemometric methods to perform multi-component determinations of drugs. Although most of the FI applications quantify a single target, interest in flow methods in multi-component analysis cannot be underestimated. Flow systems contribute to achieving high sample throughput, and minimize sample and reagent consumption in automated, simple, miniaturized procedures. Selectivity for each analyte of interest often relies on physico-chemical approaches (e.g., specific reagents, multi-parametric detection devices and multi-channel set-ups). Unfortunately, the performance of these strategies is limited and interference problems may sometimes arise. In these circumstances, chemometric methods for data analysis can be exploited to attain selectivity. In this way, FI methods can be extended to complex matrices from pharmaceutical, clinical and food fields.

Keywords: Chemometrics; Clinical; Complex matrix; Data analysis; Drug determination; Flow-injection analysis; Food; Multi-component analysis; Multivariate calibration; Pharmaceutical

Javier Saurina*
Department of Analytical Chemistry,
University of Barcelona,
Martí i Franquès 1 – 11,
08028-Barcelona,
Spain

*Tel.: +34 93 403 9778;
Fax: +34 93 402 1233;
E-mail: xavi.saurina@ub.edu

1. Introduction

The first applications of flow methods to multi-component analysis were reported in the 1980s with publication of methods for quantification of drug mixtures in aqueous samples and pharmaceuticals [1,2]. As interferences from the sample matrices were almost negligible, each compound was quantified easily from enzymatic reactions or kinetic discrimination of components. Since then, significant advances in this topic have been achieved [3,4]. Special attention has been paid to the development of strategies for obtaining selectivity as well as expanding the range of applications towards more complex matrices. The analysis of clinical, biochemical and food samples generally requires sophisticated pretreatments prior to quantification of the active compounds [5]. As a result, on-line implementation of auxiliary chemical operations (e.g., solid-phase extraction, liquid-liquid extraction, dialysis, gas-diffusion, and derivatization) becomes especially relevant [6].

Multi-component analysis by flow-injection (FI) and related flow techniques is mainly focused on the simultaneous determination of a few target compounds, generally two or three [3]. For more analytes, efforts required to develop the method may not be matched by the advantages offered by flow systems. In such circumstances, separation techniques seem to be a more reasonable choice.

In general, FI methods do not physically separate components that flow together through the system and reach the detector(s) at the same time. Hence, in the absence of separation, alternative mechanisms are needed to ensure detection of each analyte under selective conditions. For this purpose, various physico-chemical approaches, including the use of specific reagents, multi-way detectors and multi-channel manifolds, can be exploited [3]. The design of the flow-manifold, including configuration of channels as well as

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injection, reaction and detection elements, and optimization of experimental conditions are fundamental to reaching the desired selectivity. Some relevant variables often explored in this optimization are flow rates, reactor dimensions, injection volume(s) and chemical (reaction) conditions [7].

Beyond these physico-chemical-based mechanisms, application of chemometric methods can be successful when selectivity has not been achieved experimentally. Mathematical algorithms then efficiently process instrumental data [3].

2. Fundamentals of multi-component determinations

In this section, we detail the principal strategies contributing to effective discrimination of analytes in multicomponent drug determinations. First, there is a wide variety of commercially-available reagents that can be utilized for selective, sensitive labeling of analytes. Most of them have been utilized for decades in other spectroscopic, chromatographic or electrophoretic methods, and, here, they can be adapted to perform FI determinations.

Although the most common way of manipulating and delivering such reagents is in solution, the format of immobilized chemicals is of great interest in flow systems. Reagent immobilization is an attractive way to obtain additional advantages relating to the reusable nature of reactors, ease of assembly of the manifold and diminution of sample dispersion [8,9]. In general, homemade devices can be easily prepared in the format of open tubular and packed column reactors [10]. Reagents can also be immobilized on the sensing area of detectors, thus resulting in sensors and biosensors. The most important types of immobilized reagents comprise inorganic fillers (e.g., reduction agents), polymeric materials (e.g., absorbers and ion exchangers) and, especially, active biomacromolecules (e.g., enzymes) [9,11].

An important aspect to keep in mind about reactions is that, in general, each analyte has to be derivatized and/or detected independently from the others to avoid interferences. Note that, despite the formation of specific reaction products for each target compound, in practice, the resulting instrumental signals often overlap, so selective detection conditions are seldom found. As a consequence, selectivity gained through chemical reactions should be preserved during the whole process by a convenient design of the flow manifold and detection system [3].

The possibilities of flow methods in the simultaneous determination of drugs strongly depend on the capacity of detection instruments to perform mono-channel or multi-channel monitoring. A mono-channel device provides a single FI-gram consisting of a response peak over time (e.g., spectroscopy at a fix wavelength). As a result, mono-channel detectors should be utilized in combination with other instrumentation (e.g., multi-channel and multi-injection set-ups, as depicted in Fig. 1) [3].

By contrast, multi-channel or fast-scanning spectrometers record full spectra over the entire FI peak. The additional information gained through the spectral domain may contribute to increase the analytical possibilities of flow methods for the simultaneous determinations of drugs. UV-vis and fluorescence instruments are widely utilized in multi-component analysis despite the resulting spectra being unable to discriminate well. Anyway, in the absence of full spectral selectivity, chemometric methods can be applied to recover the contributions of components and to quantify the analytes on the basis of the differences in the spectral profiles.

In a similar way, fast-scanning voltammetric techniques may also be used to record full voltammograms over time, thus leading to three-dimensional FI peaks of current intensity as a function of potential and time. However, to date, the application of voltammetry to multi-component FI analysis (FIA) has been scarcely explored (see Table 1 for various recent applications). The performance of the detector is especially noticeable in the case of mass spectrometry (MS), since highly specific data for each analyte may be obtained from a proper choice of m/z traces. FIA-MS coupling can be carried out with interfaces adapted from liquid chromatography {e.g., electrospray and atmospheric pressure chemical ionization (APCI) sources [18]}.

As shown in the generic set-ups of Fig. 1, different kinds of parallel and serial configurations can be utilized to develop reactions and detection processes in the desired way. Additional channels for delivering reagents, reaction coils, flow-cells, detectors and other pieces can be added to these schemes to assemble the manifold. As a result, the versatility of flow methods is outstanding and the possibilities in constructing the manifold are almost unlimited.

In parallel configurations, each analyte is derivatized in an independent line and the resulting reaction products are then detected without interferences from the rest of derivatives (Fig. 1a and b). The design of the set-up can be modified with strategic relocation of injection port(s) and/or detection device(s). The use of multi-port rotary valves or multi-commutation devices [12] permits simultaneous injection of two (or more) sample aliquots into independent flow lines. Alternatively, splitting systems, which distribute the sample bolus into various lines, can be utilized for similar purposes. In any case, as each sample bolus follows a given channel, reactions can be developed and monitored independently.

As shown in Fig. 1b, reunifying channels prior to the detection simplifies the manifold when analytes are detected with the same technique. In this case, the confluence of all sample segments simultaneously in the