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Review

Current applications of miniaturized chromatographic and electrophoretic techniques in drug analysis

Zeineb Aturki, Anna Rocco, Silvia Rocchi, Salvatore Fanali*

Institute of Chemical Methodologies, Italian National Council of Research, Area della Ricerca di Roma I, Via Salaria km 29, 300–00015 Monterotondo (Rome), Italy

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ABSTRACT

In the last decade, miniaturized separation techniques have become greatly popular in pharmaceutical analysis. Miniaturized separation methods are increasingly utilized in all processes of drug discovery as well as quality control of pharmaceutical preparation. The great advantages presented by the analytical miniaturized techniques, including high separation efficiency and resolution, rapid analysis and minimal consumption of reagents and samples, make them an attractive alternative to the conventional chromatographic methods for drug analysis. The purpose of this review is to give a general overview of the applicability of capillary electrophoresis (CE), capillary electrochromatography (CEC) and micro/capillary/nano-liquid chromatography (micro-LC/CLC/nano-LC) for the analysis of pharmaceutical formulations, active pharmaceutical ingredients (API), drug impurity testing, chiral drug separation, determination of drugs and metabolites in biological fluids. The results concerning the use of CEC, micro-LC, CLC, and nano-LC in the period 2009–2013, while for CE, those from 2012 up to the review draft are here summarized and some specific examples are discussed.

Contents

1. Introduction

Pharmaceutical analysis is an important topic of research widely applied in several areas such as drug discovery and development, assessment of purity and quality of drug formulations, and pharmacokinetic/pharmacodynamic studies [1,2]. The high demand for analytical tools able to offer high-throughput, reliable and fast methodologies has recently stirred the interest of pharmaceutical industry towards miniaturized separation techniques, which furthermore provide reduced waste production and are cost-effective [3–5]. This attention has been additionally stimulated by "omics" research and by the introduction of protein-based drugs (e.g., monoclonal antibodies). Such applications essentially need analytical techniques capable to handle with small quantities of sample and to be easily hyphenated, i.e. with mass spectrometer (MS) [2,3,6,7].

Miniaturization of analytical and bioanalytical processes represents an important area of research which involves the implementation of several important tools such as microreactors, sampling, detection, separation systems, including the use of microchip technologies, and microextraction sample techniques [2,8,9]. Sample preparation procedures in use in many application areas are, in fact, still tedious and manually intensive protocols making the sample treatment the most time-consuming and error-prone part of the analytical scheme [10].

Taking into account miniaturized separation techniques, capillary electrophoresis (CE), capillary electrochromatography (CEC)

* Corresponding author. Tel.: +39 06 9067256; fax: +39 0690672269.
E-mail address: salvatore.fanali@cnr.it (S. Fanali).

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and micro/capillary/nano-liquid chromatography (micro-LC, CLC, nano-LC) are those largely promoted, in some instance especially from research laboratories, as valid and convenient alternative to conventional and commonly employed chromatographic techniques such as HPLC and GC. As briefly above mentioned, the common advantages making these methodologies attractive are: (i) the reduced use of reagents, including organic solvents, with consequent decreased environmental pollution and cost; (ii) the need of small sample volumes; (iii) short analysis time; (iv) rapid optimization of experimental conditions, comprising the possibility to perform experiments in parallel [3,5,11].

Considering the low flow rate of miniaturized techniques, they are particularly suitable for the coupling with MS without using split flow system. Hyphenated MS techniques are essential for an unequivocal identification of compounds and their structural analysis, possess enhanced selectivity and sensitivity. However it should be mentioned that laboratory-made interfaces often offer better performances than commercially available ones, and especially relating to CEC this coupling still results in experimental complexity [12–14].

Another drawback related to miniaturized techniques can be the low sample loading due to the small dimension of the separation medium and the low sensitivity related to on-column detection with UV detectors. However, it is possible to resort to different ruses to avoid such an inconvenience, for example on-line focusing and stacking approaches for chromatographic and electrophoretic techniques, respectively, in addition to sample preparation techniques allowing the simultaneous enrichment and purification of the analytes. Being involved the analysis of biological matrices, sample preparation represents a mandatory task for miniaturized techniques, as well, where is of paramount importance to avoid system overloading and/or clocking [8,15–20].

Comparing CE with CEC and nano-LC/CLC, the first one is of easier implementation in relation to the separation medium. In fact, few kinds of packed capillary columns (monolithic or particulate) are commercially available for miniaturized version of chromatography and CEC. As a consequence, most research laboratories prepare themselves packed capillary columns in order to amplify the range of selectivity, increase efficiency and speed up analysis, by the use of innovative stationary phases. Another restriction related to CEC is the need of stationary phases able to generate an electroosmotic flow (EOF); this can be easily solved utilizing material that contains charged or chargeable groups [5].

In this review all these aspects will be covered considering the applications of miniaturized separation techniques in the different branches of pharmaceutical field. In this contest, especially noteworthy is the role played by miniaturized techniques in chiral drug analysis [4,21,22] to which a specific section is dedicated. Finally, the implementation of such techniques as laboratory-on-chip for pharmaceutical analysis is also discussed.

Considering the previous published review papers [1,3–5,23,24], this one takes into consideration the applications of CEC, micro-LC, CLC, and nano-LC in the period 2009–2013, while for CE, those from 2012 up to the review draft.

2. Analysis of pharmaceutical drugs by capillary electrophoresis

Since its early application in drug analysis [25,26], CE has become an established routine analytical method and together with HPLC, is the most used separation technique in pharmaceutical field. Remarkable features, such as high efficiency, great resolution power, rapid analysis, automation, low sample consumption and low costs as well as the hyphenation with MS detectors, make CE an increasing popular analytical tool for pharmaceuticals analysis. Additionally, the development of various modes of CE including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC), non-aqueous capillary electrophoresis, (NACE), isotachophoresis (ITP), isoelectric focusing (IEF) allows the analysis of ionic and non ionic compounds, drugs poorly soluble in water, etc.

One of the most mentioned limitations of electrophoretic separation methods when compared to traditional liquid phase separation techniques such as HPLC is the lower concentration sensitivity.

To overcome this problem, many approaches including on-line and off-line pre-concentration techniques have been developed. Breadmore et al. have published a series of reviews concerning the field amplified sample stacking, large volume sample stacking, dynamic pH junction and sweeping methods [27]. The coupling with alternative detection systems instead of the common UV absorbance detectors was also considered to achieve a higher method sensitivity. The hyphenation of CE with MS detectors allows getting lower limits of detection, of great importance for the analysis of drugs and metabolites in biological fluids and for drug impurity profiling. Furthermore, the use of MS detectors provides the advantage to obtain the unambiguous characterization of the analyzed drugs in complex matrices [28,29]. Fluorescence (FL) and more recently contactless conductivity detectors (C^4D) have been often used, especially the last one mentioned for their compatibility with the miniaturized techniques [30,31].

In recent years, in addition to chromatographic methodologies, CE has become a useful orthogonal separation technique to HPLC in drug development, quality control, as well as in impurity profiling and in drug discovery [32–34].

The following section illustrates the analysis of drugs in pharmaceuticals and biological fluids by CE covering the literature published in the last two years, reporting the data in Table 1.

The works described have been selected on the basis of the novelties concerning the methodological improvement and relevant applications.

An important aspect to highlight in CE is presented by the different approaches utilized to obtain sensitivity enhancement of the technique. As follows, some works employing on-line preconcentration strategies are reported.

The simultaneous determination of tricyclic psychiatric drugs in urine samples by CE was reported by Dziomba et al. [35]. With the purpose to obtain a significant sensitivity improvement, an on-line pre-concentration approach based on micelle to solvent stacking (MSS) was optimized. Several experimental parameters, such as concentration and pH buffer, organic solvent content, SDS concentration, applied voltage and injection volume, were investigated to achieve optimum separation efficiency and highest sensitivity method. The developed MSS-CE method allowed the determination of the analyzed psychotropic drugs at ng/ml level in human urine. LODs values achieved were about 50 folds lower with respect to a conventional CE method. This study represents a very useful application because the quantification of psychiatric drugs in biological fluids is needed for therapeutic drug monitoring, pharmacokinetic studies, and quality control of dosage forms.

A selective and sensitive CE method for the determination of ephedrine and pseudoephedrine in human urine and serum was proposed. A monolithic molecular imprinted polymer (MIP) based solid phase microextraction (SPME) was developed for the concentration and clean-up of the two drugs from biological fluids. The MIP fibers for SPME were prepared using ephedrine as template by an *in situ* polymerization method using a silica capillary as mold. MIP-SPME-CE system allowed detecting the two alkaloids at high sensitivity with LODs values of 0.96 and 1.1 ng/mL for ephedrine and pseudoephedrine. With this method, a sensitivity improvement of