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Changing scenario of pharmaceutical analysis

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ABSTRACT

The authors outline the trends in the field of pharmaceutical analysis in the past 25 years. The most remarkable development has been the introduction and rapid spread of high-performance liquid chromatography. This technique has become the most important method in the quality control of bulk drugs and pharmaceutical formulations, even at the pharmacopoeial level, and (increasingly coupled with mass spectrometry) also in the determination of drugs and metabolites in biological samples. The changes in the role of other chromatographic (electrophoretic) and spectroscopic techniques are also discussed with emphasis on their hyphenated variants. There are separate sections devoted to chiral issues and regulatory aspects of drug analysis with emphasis on questions related to validation.

KEYWORDS

Pharmaceutical analysis;
Chromatography;
Spectroscopy;
Drug.

1. INTRODUCTION

The scope of drug analysis includes the analytical investigation of bulk-drug materials, the intermediates in their synthesis, products of drug research (potential pharmacons), drug formulations, impurities and degradation products of drugs, biological samples containing the drugs and their metabolites with the aim of obtaining data that can contribute to the maximal efficacy and maximal safety of drug therapy and the maximal economy of the production of drugs. The efficacy, safety and economy of drug therapy are extremely important issues not only from the point of view of public health, but their financial, moreover political, aspects are also immense. As a consequence of this, pharmaceutical and biomedical analysis is among the most important branches of applied analytical chemistry. To fulfill the rapidly increasing demands as regards the number and the quality of analytical measurements, great efforts have been made and are being made to apply, moreover further develop in this field, the latest achievements of analytical chemistry. This can be demonstrated by the great number of books devoted to this topic in the last quarter of century, and up to the present time[1-10]. The importance of drug impurity stability- related issues can also be characterized by the large number of books devoted to this subject[11-15]. The determination of drugs and metabolites in biological samples[16-21], with particular attention to toxicological and forensic analysis[22-31], requires special techniques and special ways of thinking, as reflected by many books on these issues. Drug discovery requires a solid analytical background with a great variety of methods to be used[32,33] (and several more references in the sections below). Reference will be made to several more books in the sections below dealing with various methodological branches of pharmaceutical analysis and other issues.

2. The HPLC Era

The most characteristic feature of the development in the methodology in pharmaceutical and biomedical analysis in the past 25 years is that various forms of high performance liquid chromatography (HPLC) be-

Review

came undoubtedly the most important method[34-37]. The theoretical and practical foundations of this method were laid down at the end of 1960s and at the beginning of 1970s. The latter decade was the period that saw the rapid spread of this technique. We can try to characterize the position of HPLC in pharmaceutical analysis at the beginning of the 1980s by the share of the various techniques in the 1st Volume of the Journal of Pharmaceutical and Biomedical Analysis (JPBA, 1983). In no less than 50% of the papers in this volume, HPLC was applied for the solution of various problems, leaving the other 50% to about 15 other chromatographic, spectroscopic and other methods-about 10% gas chromatography (GC), 5% thin-layer chromatography (TLC), 10-10% ultraviolet (UV) spectrophotometry and electroanalytical methods. This case applies only partly to pharmacopoeias, which are naturally more conservative than the authors of current research articles. HPLC appeared for the first time in the 20th revision of the United States Pharmacopoeia with much more modest but yet remarkable contribution (about 5% in the assays of bulk drugs and drug formulations, respectively, and with a few examples at that time of chromatographic purity tests that occasionally featured in some monographs)[38]. The contribution of HPLC in the recent Volumes 38 and 39 of JPBA has further increased to about 65%. However, a great difference is that, while in 1983, a UV detector was used almost exclusively (leaving a little share to refractive index, fluorimetric and electrochemical detection), in 2005, in about one third of the HPLC papers, a mass spectrometer was used as the detector. Due to its high sensitivity and selectivity, HPLC coupled with mass spectrometry, HPLC/MS (MS) or LC/MS (MS) have become the predominant method in bioassays, and pharmacokinetic and metabolic studies, as well as in the structure elucidation of drug impurities and degradation products[39,40]. The breakthrough of HPLC in compendial analysis of small organic molecules was also extremely rapid. In the latest (29th) revision of the United States Pharmacopoeia[41], HPLC is used for the assay of bulk-drug materials of this type in about 45% of the monographs. This share is somewhat higher than that of the non-selective but much less time-consuming titration methods, leaving only about 10% to other methods, mainly the similarly non-selective UV-Vis spectrophotometry. As for the purity control of bulk-drug materials (related compounds test, which has become quite general since 1980), HPLC and TLC are used almost exclusively with about equal shares. Even more spectacular is the propagation of HPLC in the assay of pharmaceutical formulations, which need specific, stability-indicating methods: a share of about 75%. No other method has spread so rapidly in the history of pharmaceutical analysis. In addition to the spread of HPLC/MS, another new development in this field has been the introduction of column packings with ultrafine particles (<2 lm) enabling short columns (5 cm or less) to be used and rapid analyses (e.g., 5 min or even less than 1 min) to be carried out (by UPLC = ultra performance liquid chromatography)

3. Other chromatographic methods[42-45]

The most important application field of modern TLC is the separation of the components of complex mixtures (e.g., impurities and degradation products of drug materials, and extracts of medicinal plants). The speed and the resolution could be greatly improved by the introduction of special techniques, such as high performance thin-layer chromatography (HPTLC) using ultrathin layers and coatings with ultrafine particles or Over pressured-layer chromatography (OPLC). The development of densitometers enables classical TLC and the latter techniques to be successfully used as tools for the quantitative analysis of complex mixtures. As a consequence of the introduction and rapid spread of HPLC (and HPLC/MS), the importance of GC (and GC/MS) naturally decreased somewhat. Nevertheless, these are still important techniques in many fields of drug analysis where the analytes are volatile and thermally stable enough. Moreover, a new field of application in the past 15-20 years became the determination of residual solvents in drugs. Almost exclusively, the headspace technique is used to enable the demanding requirements to be fulfilled (i.e. determination of the solvents at the 10-ppm level; moreover; in the case of carcinogenic or genotoxic solvents, down to the ppm level).

4. Capillary electrophoresis and related techniques[46-48]

Since the introduction of the first commercially available instruments in the late 1980s, capillary electrophoresis (CE) and related methods, such as micellar Electrokinetic chromatography (MEKC), microemulsion Electrokinetic chromatography (MEEKC) and capillary

electrochromatography (CEC)[48], have attracted great interest in pharmaceutical analysis as possible alternatives or amendments to HPLC. This trend can be characterized by their remarkably high share (about 10%) in the papers in the 2005 volumes of JPBA. On the one hand, this share is an underestimate, since there are journals specializing in CE. On the other hand, it is an overestimate, since, despite CE having several advantages, such as a flat flow profile, that result in an extremely high column efficiency, due to limitations as regards its general applicability, it does not yet seem to be a real rival to HPLC in the practice of compendial industrial pharmaceutical analysis. CE is already an official method in USP XXIX[41], but its contribution to the monographs is still negligibly low. However, it is already an inevitable tool in the analysis of proteins and other biopolymers with particular respect to miniaturization leading to chip-based bioanalytical chemistry. Of the new techniques mentioned above, CEC will certainly have a bright future.

5. Spectroscopic methods and their hyphenated variants

The development of nuclear magnetic resonance (NMR) and mass spectrometry (MS) in the past 25 years, along a road paved with Nobel Prizes, has also been successfully exploited in pharmaceutical and biomedical analysis. In particular, the dramatic decrease in the demanding requirements for sample size and the solution of the difficult problems of interfacing these techniques with chromatographic (and electrophoretic) separation methods have greatly expanded their field of application. In addition to the off-line applications that are still widely used, on-line HPLC/MS, HPLC/NMR and HPLC/NMR/MS, and other hyphenated methods, are becoming the leading methods in, for example, the structure elucidation of drug impurities, degradation products, metabolites, and bioactive components in natural products[49,50]. Due to its high sensitivity and selectivity, HPLC/MS (MS) has become the predominant method, even in the quantitation of these minor components (e.g., in pharmacokinetic and bioequivalence studies). A renaissance of UV spectroscopy[51] is observable due to the availability of diode-array detectors attached to HPLC and TLC densitometers, both suitable for obtaining good-quality spectra, which are often useful, for example, in identifying impurities. As for the quantitative analytical application of this technique, the approximately 10% share in pharmacopoeias for the assay of bulk-drug materials and pharmaceutical formulations is only very slowly decreasing. Derivative spectrophotometry and multiwavelength/chemometric measurements were interesting and successful research areas at the beginning of the 25-year period. At present, these can be considered to be fairly important routine methods. It is remarkable that, in the course of the past 25 years, colorimetric methods based on chemical reactions have almost entirely lost their importance. The most important application of fluorimetry in modern pharmaceutical and biomedical analysis is as detectors attached to HPLC or related techniques. In particular, laser-induced fluorimetry based on native or derivatization-based fluorescence enables very sensitive determinations to be carried out. The most important field of application of infrared (IR)[52] and near-infrared (NIR) spectroscopy[53] is the identification of drugs. IR has greatly decreased (almost completely eliminated) the importance of the classical color tests, while NIR is a method of increasing importance in the in-process control in manufacturing pharmaceutical formulations. IR and Raman spectroscopy, together with solid-phase NMR, X-ray diffraction and thermal methods are the up-to-date methods in solidphase characterization[54], which is of great importance in developing pharmaceutical formulations with optimal bioavailability. Although the classical sulfide and other limit tests are still widely used in pharmacopoeias for (toxic) metal impurities, the importance of the much more selective and sensitive atomic spectroscopic methods, such as graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma atomic emission spectrometry (ICP/AES) and mass spectrometry (ICP/ MS), is rapidly increasing.

6. Other methods

Although titrations are non-selective, this classical method is still widely used in compendial analysis for the assay of bulk-drug materials. Its share in the European Pharmacopoeia[55] is almost 70%. Even in the USP, where the breakthrough of HPLC has been much faster, more than 40% of low molecular weight organic compounds are determined by aqueous or non-aqueous titration. It is remarkable that potentiometric endpoint detection step by step replaces the use of indicators (especially in the European Pharmacopoeia). Other

Review

electroanalytical methods have always been only modestly important in pharmaceutical analysis. Classical polarographic methods using toxic mercury electrodes are being driven out from practice and replaced by new electrodes (e.g., glassy carbon electrodes modified with carbon nanotubes enable highly sensitive analyses). Another field where remarkable results have been obtained is the development of ion-specific and moleculespecific sensors. Flow-injection analysis with various spectroscopic and electroanalytical chemiluminescence detectors is often used in the analysis of drug formulations[56]. Twenty-five years ago, all antibiotics were determined by microbiological methods[57]. In the modern pharmacopoeias, in the majority of cases, these are replaced by much more selective and informative methods, mainly HPLC. Although the importance of immunoassays has decreased in the recent years, they are still often used in the determination of some bioactive compounds in biological samples. Radioimmunoassay has been greatly superseded by various enzyme-immunoassay methods.

7. Chiral issues

The separation and the quantification of enantiomeric mixtures are among the great challenges of the past quarter-of-a-century in pharmaceutical and biomedical analysis[58-65]. The main problems to be solved are the determination of the enantiomeric purity of drugs being used in therapy as pure enantiomers and the simultaneous determination of the components of racemates in biological samples. In the beginning, GC separations based on homochiral derivatization followed by separation of the forming diastereomeric pair on achiral stationary phases and direct determinations on chiral stationary phases were the predominant methods. Later on, HPLC replaced GC in both branches of enantiomeric separations, and also enabled the use of a third approach: dynamic formation of diastereomeric adducts using homochiral mobilephase additives and their separation on achiral stationary phases. The latter type of enantiomeric separation has also been successfully adapted to CE[65]. The present situation can be characterized by the spread of this technique and the continuous development and commercialization of new types of chiral HPLC columns.

8. Regulatory issues: the present situation and future trends[66-69]

Due to the globalization of the drug market and the sharpening concurrence among the drug companies, pharmaceutical analysis has become one of the battlefields in the struggle. The importance of issues related to drug safety has greatly increased and this has led to the continual increase of demands as regards securing the quality of drugs, moreover often over securing the safety of drug therapy. To fulfill the above requirements, it became necessary to harmonize the demands and analytical strategies. The first step was establishment in the early 1970s of the European Pharmacopoeia, of which the 5th Edition is now official[55]. This became the basis of the national pharmacopoeias of the member states of the European Union. The next step was formation of ICH (International Conference on Harmonization) in 1990, with the aim of harmonizing the efforts of registration agencies, principal pharmacopoeias (Ph. Eur., USP and Japanese Pharmacopoeia) and pharmaceutical manufacturers organizations to improve the quality of drugs and the safety and efficacy of drug therapy. The guidelines issued by ICH are authoritative worldwide with respect to drug-quality issues. However, it has to be noted that the requirements as regards the quality of the drugs and drug formulations in the drug market are in practice much greater than those prescribed in the pharmacopoeias and ICH guidelines. The most remarkable change in the pharmacopoeias in the past 25 years has been the increasing importance of purity tests. At the beginning of this period, only a very limited number of monographs contained tests related to impurities. Thanks to the development of TLC and HPLC, at present, the overwhelming majority of the monographs on bulk drugs and in a fairly high proportion of those on formulations contain these tests. The impurity profile has become the most informative indicator of the quality of bulk-drug materials. At the same time, the importance of assaying bulk drugs has decreased considerably; moreover, there are opinions that even this importance is questionable[70]. As a consequence of the tendencies towards globalization and harmonization mentioned above and the necessity of increasing the safety of drug therapy, validation of the analytical methods has come to the forefront; moreover, it has become one of the most important issues in contemporary drug analysis. The authors of this article have no doubt about the importance and the necessity of validating analytical methods in pharmaceutical analysis. However, some negative tendencies are also ap-

parent: a fully validated method meeting the requirements of various guidelines needs much more analytical data than would be strictly necessary; as a consequence of this, many drug analysts (especially among the young generation) feel that the essence of pharmaceutical analysis is the mass production and handling of data rather than problem solving; and, the way of thinking is changing, with many people (mainly outside the circles of drug analysts) believing that possession of up-to-date, automated/computerized (and very expensive) instruments and validated methods automatically give good and reliable results. In contrast to the above concerns, the authors wishes to conclude this article by expressing their, perhaps somewhat old-fashioned, view: pharmaceutical analysis, even at the beginning of the twenty-first century, is not only an important field of activity in the interest of suffering mankind through increasing the safety of drug therapy but it is also at the same time a source of inexhaustible intellectual pleasures.

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Review

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