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Analytical Survey

Chemometrics in pharmaceutical analysis

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Introduction

This survey examines the chemometric methods that have or could have an effect on the way pharmaceutical analysis is carried out. The review has been deliberately limited to the techniques most commonly used in pharmaceutical analysis (spectrometry, chromatography and a few others) since the authors seek to demonstrate that chemometric methods can be applied in even the most humble of routine pharmaceutical analytical laboratories. The definition of what constitutes a chemometric method has been interpreted rather liberally for this review, and covers almost any application of statistical or numerical methods to pharmaceutical analysis.

Textbooks and Reviews

In recent years there has been a gradual increase in the number of textbooks on, and around the periphery of, the subject of chemometrics [1–19]. None of these books is aimed specifically at pharmaceutical analysis, but even a superficial reading would suffice to demonstrate the potential applications of chemometrics in this field.

A basic understanding of statistics can be obtained from the excellent books by Caulcutt and Boddy [15] and Miller and Miller [16], the latter of which includes an introduction to chemometric methods of optimisation and pattern recognition. The book by Massart *et al.* [1] remains the standard textbook on chemometrics and is essential reading for all student chemometricians. The text written by Davis [14] is now rather old (written in 1973) and is aimed primarily at geologists, but it remains one of the most lucid explanations of chemometric methods, leading the reader gently through the complexities of matrix algebra and assuming no previous mathematical experience beyond normal school education. Davis has written the book in a very readable style and includes well annotated computer programmes and easily understood examples. The two

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books by Kowalski [4, 9], who is rightly acknowledged as the "father of chemometrics", provide good overviews of the scope of chemometrics and its applications. For those particularly interested in pattern recognition, principal component analysis, factor analysis and cluster analysis there are three excellent texts available [2, 3, 5], all of which have found great use in the authors' laboratories. The *magnum opus* by Jambu and Lebeaux [17] on cluster analysis is not for the beginner, but is an excellent reference work. The three books on transform methods [6–8] require of the reader a fair degree of mathematical ability, but are, nevertheless, worth the effort, since the techniques and examples described show both the fundamentals and power of transform techniques. The text by Everitt [19] is not specifically concerned with chemometrics, but does contain some useful hints on data presentation and is easy reading. The two books by Eckschlager and Stepanek [10, 11] examine chemical analysis as a problem in information theory. The volume by Wolff and Parsons [13] is a good guide to the commercial software packages available, but is aimed at mainframe and minicomputer, rather than microcomputer, users. The book by Balke [12] looks at the applications of chemometrics in the field of liquid chromatography (LC), and therefore should be of great benefit to pharmaceutical analysts.

Chemometrics is at the fortunate stage of development where all of the currently available books are of excellent quality and it is hoped that there will not be a proliferation of poor quality volumes as publishers attempt to profit from the growth of the science.

The biennial "Fundamental Reviews" in *Analytical Chemistry* now include a Chemometrics section [20–22] and provide an excellent update of the work in chemometrics during the preceding two years. From these reviews it is simple to select the papers relevant to pharmaceutical analysis. The Chemometrics Society issues periodic bulletins to its members which often contain updated lists of publications in chemometrics. Since the application of chemometrics necessarily requires the use of computers, the periodic "A/C Interface" articles by Dessy [23–41] in *Analytical Chemistry* provide a useful means for the analyst to keep abreast of pertinent computer technology.

General Experimental Design and Data Analysis

The design of an experiment and the analysis of the data depend on the objectives to be achieved. This part of the survey reviews some of the published techniques for both operations.

Experimental design

One of the common operations carried out in pharmaceutical analysis is the collaborative trial to evaluate a new analytical method. The classical work of Youden and Steiner [42] contains much useful advice on collaborative experiments and the value of using a ruggedness test before subjecting a method to a collaborative trial. Two British Standards [43, 44] on collaborative trials have been published. Although one of them [44] is aimed primarily at the petrochemical industry, the principles remain the same whatever the analyte and matrix. The use of these in pharmaceutical analysis has been discussed by Fisher [45].

The generalised standard addition method (GSAM) was developed by Saxberg and Kowalski [46] and simultaneously corrects for matrix effects and interferences in multi-

component analysis. This has been developed by other workers (e.g. [47, 48]) and used in many techniques.

Simplex designs and their application in analytical chemistry were developed mainly by Deming and co-workers, who have reviewed these applications [49–51]. Further development of the method has produced the super-modified simplex [52] and the high speed simplex [53]. The simplex method is an easily understood method for the simultaneous optimisation of several variables that may affect the response of an analytical system. For simple systems (less than three variables) it can be used graphically without resort to a computer.

Data analysis

The abstraction of meaningful information from large sets of multivariate data is regarded by many as the true field of chemometrics. The most common type of data analysis in pharmaceutical analysis is probably still the fitting of a straight or curved line through a set of data points. The classic work on this subject has for many years been that by Draper and Smith [54]. The application of such methods has been examined by Hunter [55] in the analysis of F D & C Red No. 2 by high-performance liquid chromatography (HPLC). A great deal of attention has been paid to the problem of curve fitting and Schwartz has produced an elegant solution to the problem using orthogonal polynomial methods [56–58]. An excellent paper by Deming [59] discusses the problem of model fitting to experimental data, and gives lucid insight into the problems and parameters involved.

To abstract meaningful information from large multivariate data sets, the techniques of principal components analysis (factor analysis) and cluster analysis have been developed, and the books discussed earlier are devoted to these subjects. Various other methods have also been developed including SIMCA [60–62] and PRIMA [63]. DISCLOSE [64] was written for dealing with chemical and pharmaceutical data. Developments in computer graphics have made it possible to easily visualise these large multivariate sets and their fitted models [65, 66], and as 16 and 32 bit computers with Mbytes of RAM become available, the bench analyst should soon find very sophisticated data displays becoming cheaply and readily available.

The use of Kalman filtering for the rapid solving of multicomponent problems is receiving a great deal of attention [67–69], and should be of benefit to pharmaceutical analysis.

Chromatography

Chromatographic methods are the most widely used analytical techniques in pharmaceutical analysis, and this part of the survey is restricted to the three most popular areas, HPLC, thin-layer chromatography (TLC) and gas–liquid chromatography (GLC).

High-performance liquid chromatography

The applications of chemometrics in HPLC are in two main areas: (i) optimisation of separations and (ii) data handling and manipulation.

Optimisation of separations. Formalised methods for optimising separations began to take shape with the early work of Snyder and co-workers [70], and were made more accessible to most analysts by Berridge [71] who programmed the method for a T159

programmable calculator. From this genesis, two main methods of optimisation have matured, the solvent selectivity–triangle method, and the simplex optimisation method. The solvent selectivity–triangle method was developed by Glajch and co-workers [72–75] at du Pont, and added to by others (e.g. ref. 76). The method uses known properties of the organic solvents used in HPLC mobile phases and combines the solvent selectivity–triangle concept with a mixture–design statistical technique to optimise the mobile phase and utilises overlapping resolution mapping to display the results in an easily interpreted form. Prior to du Pont's withdrawal from the HPLC hardware market, the system was marketed by them under the name of SENTINEL. The method has the disadvantage in that it optimises only the solvent composition of the mobile phase and does not permit simultaneous optimisation of the pH, salt concentration, ion-pairing agent concentration, flow rate, column temperature or any other parameter that may contribute to chromatographic separation. Simplex optimisation does allow simultaneous optimisation of all of these parameters, but is a "brute force" method that takes no account of any known theoretical aspects of the chromatographic process. Watson and Carr used the method in 1979 [77] and it has been further developed and fully automated by Berridge [78–82] using standard equipment from Laboratory Data Control (LDC). Both the solvent selectivity–triangle method and the simplex optimisation method, with the correct hardware and software, permit completely automatic, unattended optimisation of chromatographic separations.

Reviews of HPLC optimisation methods have been published recently by Berridge [83] and D'Agostino *et al.* [84]. Berridge's review also includes a discussion of the currently less popular window-diagram method [85]. Debets *et al.* [86, 87] have reviewed the various criteria used in judging optimum separation.

Commercial packages for carrying out on-line, unattended optimisations of HPLC separations are available from LDC (TAMED), Spectra Physics (OPTIM), Bruker and Perkin–Elmer.

Examples of applications of optimisation methods include the separations of pilocarpine and isopilocarpine in ophthalmic formulations [88], phenothiazines [89], steroids [90, 91], caffeine, saccharin and benzoic acid [92], morphine, codeine, noscapine and papaverine [93], and beclomethasone dipropionate and its decomposition products [94].

For dealing with the problem of the non-equivalence of supposedly identical LC columns from different manufacturers, Smith has compared columns using cluster analysis [95].

Data handling and manipulation. Prior to the advent of diode array detectors, the only real applications of chemometrics in data manipulation in LC were in the fields of resolution enhancement and peak deconvolution, and the applications of Fourier transform methods involving cross correlation, auto correlation and deconvolution have been reviewed by Malczewski and Grushka [96] and Annino [97]. The use of correlation chromatography [114, 115], which utilises these techniques, has, as yet, found little use in pharmaceutical analysis. By using stopped-flow scanning Halket [98] used factor analysis to determine peak purity in chromatograms obtained by HPLC. Similarly McCue and Malinowski [99] collected fractions during the chromatography, measured post-run spectra and then used rank annihilation factor analysis to measure unresolved peaks. Doornbos, Smit and co-workers [100, 101] have used orthogonal polynomials to deconvolute chromatograms and have illustrated the method on a mixture of five sulphonamides.

The introduction of computerised photodiode array UV–visible detectors in HPLC has permitted a great increase in the use of chemometrics for obtaining useful information from the absorbance (A)–wavelength(λ)–time(t) matrices generated by these instruments. With the rapid on-line data capture and computer facilities it is possible to use either second derivative (d^2A/dt^2) or absorbance ratio methods to ensure peak homogeneity [102–107]. The spectral suppression method of Fell and co-workers [104] provides a novel and elegant method for determining one component in a chromatographic peak that consists of a number of co-eluting components. The progress in computer graphics has allowed many new ways of presenting the data in the A–λ–t matrices [106, 108, 109]. Initial work on archive retrieval systems has been reported [110], and it can be expected that this work will be developed so that fast on-line identification of chromatographic peaks will be achieved. Osten and Kowalski [111] have used factor analysis of the A–λ–t matrix to resolve and quantitate overlapping chromatographic peaks, and this standard piece of software is now commercially available from Infometrix as the MCR2 package for the Hewlett–Packard 1040 diode array detector. Other workers have developed this method further using factor analysis [112] and target factor analysis [113]. Frans and co-workers [116] have used reiterative least-squares resolution to resolve up to seven peaks, but make some assumptions about peak shape and use computing power beyond the reach of many pharmaceutical analysts.

Otto *et al.* [117] have made use of a combination of principal components analysis and multiple linear regression with the partial least-squares method to quantify components under overlapping chromatographic peaks, regressing the principal components on the spectra of the two or more pure compounds.

Many of these techniques have been used in the field of pharmaceutical analysis, for the measurement of noscapine and papaverine [104], diacetylmorphine, 6-acetylmorphine and morphine [105], promethazine hydrochloride and its sulphoxide impurity [109], diphenhydramine in formulations [109], zimeldine and its metabolites [106, 109], amino acids and peptides [108], diacetylmorphine, caffeine, papaverine, 6-acetylcodeine, thebaine, 6-acetyl morphine, procaine and morphine [102], and for the identification of 8-chlorotheophylline, cortisone acetate, cycloserine, ethinyloestradiol, ethisterone, progesterone, theophylline, caffeine, theobromine and morphine [110].

Thin-layer chromatography

Probably due to the fact that TLC is much more a manual rather than an instrumental technique, there has been far less application of chemometrics to TLC than to other chromatographic techniques. As with HPLC, the simplex [118], overlapping resolution mapping [119] and window-diagram [120] methods have all been applied to the optimisation of TLC mobile phases. Ebel and co-workers have used statistical methods to evaluate the errors in the quantitative TLC of dyes and paracetamol [121, 122]. Musumarra and co-workers have applied principal components analysis to the TLC data for five hundred and ninety-six basic and neutral drugs in four eluent systems [123, 124]. The same workers also used principal components analysis in examining the Rf values of amines in chloroform–ethyl acetate mixtures [125].

Gas–liquid chromatography

Obviously some of the chemometric techniques used in HPLC can also be applied to GLC. Following the pioneering work of Morgan and Deming [126], simplex optimisation has been applied to GLC by many workers [127–133]. Van Hare and Rogers have

examined the information contents of separation functions used in optimisation procedures [134]. Linnett and Atkinson [135] used differentiation of the chromatogram to enhance narrow peaks and suppress broad peaks in the analysis of *n*-alkanes. Pattern recognition techniques have been applied to abstract meaningful information from large sets of GLC data [136–141].

Spectrophotometry

Spectrophotometric methods traditionally have been restricted to the simpler problems in pharmaceutical analysis. Although techniques for improving specificity have been known for some years, it is only the availability of spectrophotometers with data systems to acquire and process the data rapidly which has made them accessible to the practising analyst. This section of the review deals with the main spectrophotometric techniques of UV and IR spectrometry and fluorescence spectrometry.

Ultraviolet spectrometry

Ultraviolet spectra are usually broad and lacking in fine detail, necessitating some form of data handling to analyse mixtures or to remove interferences from excipients. The most popular method at present is derivative spectrometry, which differentiates the absorbance spectrum with respect to time or wavelength. Differentiation may be carried out either with an electronic module which processes the analogue output from the spectrometer or by manipulation of the digitised spectrum. Electronic modules produce a wavelength shift in the spectrum, which is no disadvantage since the test and standard are treated similarly. Digital derivatives are often obtained by the least-squares polynomial method of Savitsky and Golay [142, 143] or the simple wavelength difference method of Butler and Hopkins [144]. A useful introduction to the technique is given by Cahill [145]. The effect of derivative spectrometry is to accentuate the narrowest peaks in a spectrum. Its usefulness in resolving overlapping peaks has been studied theoretically [146]. The applications to pharmaceutical analysis were pioneered by Fell [147]. Second derivative spectra have proved most useful for quantitative analysis. Examples include the assay of diphenhydramine in a complex formulation [148], the assay and identification of dyes in formulated products [149] and a number of applications to drugs with weak benzenoid absorption [150–153]. Most modern scanning spectrometers have facilities for obtaining derivative spectra. When either selecting a spectrometer or developing a method it should be remembered that differentiating the spectrum decreases the signal-to-noise ratio, so that it may be necessary to carry out replicate measurements for each solution to obtain the required assay precision.

Another method of data treatment is the use of orthogonal polynomials. The spectrum is digitised with a fixed wavelength interval and fitted by a sum of orthogonal polynomials of different orders. The concentration of the compound of interest is proportional to the coefficient of a selected polynomial [154] or, in the combined polynomial method, to a combination of coefficients of different polynomials [155]. Like derivative spectrometry the method will remove interferences from other drugs or from excipients which absorb or scatter the incident light. It has been used to remove interferences from degradation products in the stability assays of formulations [156, 157]. Suitable calculation facilities are not generally available for commercial spectrometers with data systems, but the calculations can be programmed in Basic and carried out on microcomputers.

Two major methods are available for statistically fitting standard spectra to a test spectrum: the least-squares method and the Kalman filter. For both methods in their original form it is necessary to have spectra of all the absorbing species. However, prior differentiation of the data may be used to remove broad band interferences. The least-squares method treats the data set as a whole and minimises the squared residuals [158]. The number of wavelengths exceeds the number of component spectra. Modifications of the basic method include non-negative linear least-squares [159] and the P matrix method [160] which allows for interactions between the components at the cost of extra measurements. Applications have been reported for liquid and solid products [161, 148] and for amino acids [162]. Calculation facilities for the least-squares method are available on a number of modern spectrometer systems. They often use mixed standard solutions, which correct to some extent deviations from Beers law, though this is less of a problem with UV spectrometry than with IR. The calculations can also be programmed in Basic for microcomputers, the only difficulty being the need for a matrix inversion.

The Kalman filter has received a lot of attention recently. It is a recursive method and requires initial estimates for the concentrations, which are then refined by using the data for each wavelength in turn [163]. It has been suggested that this procedure would be particularly useful for computer-controlled spectrometers because the measurement process can be continued until the estimated concentrations are constant [163]. However, the method is not available on a commercial system as yet. The calculations are simpler than for the normal (non-recursive) least-squares method. Criteria for optimising wavelength selection in both methods have been discussed [164–167]. Recent papers have discussed multicomponent analysis in the presence of interferences [69, 168]. Under certain limiting conditions it is possible to determine the concentrations of the analytes.

Fourier transform techniques are also capable of many operations on spectra such as smoothing, differentiation, resolution enhancement, cross-correlation and self-deconvolution [169–172]. These methods are not available for commercial spectrometer systems at present.

Infrared spectrometry

Quantitative IR spectrometry starts from a two-dimensional data matrix, absorbance or transmittance versus wavelength or wavenumber, in the same way as UV spectrometry and the same methods of data treatment are applicable. However, the differences in solvents and sample preparation, together with the much sharper bands, mean that techniques such as differentiation to remove background absorption are less important. The book edited by Mattson, Mark and Macdonald gives a useful introduction to computing aspects of IR spectrometry [173].

A recent review by Brown has focused particularly on multicomponent analysis [174]. The least-squares method is popular and is available for several commercial spectrometer systems. IR spectrometry is prone to problems of non-linear response due to interaction with the solvent or other components in solution. Modifications of the least-squares method or the related P matrix method have been made to compensate for non-linearity [175, 176]. Brown has described the optimisation of such an analysis for mixtures of lipids [174]. Some interesting applications of data handling have been developed for near IR spectrometry (NIR). NIR measurements are often made by reflectance on solid samples, and the bands are more similar in shape to those in UV

spectra. Fourier coefficients, which have been used for quantitative analysis of tobacco samples, permitted severe compression of the data before calculation [177].

Qualitative analysis by IR spectrometry often involves library searching. Whilst chemometric techniques may be involved in the search programmes, it is usual for the analyst to purchase a package from the manufacturer of the instrument or from a software house. The detailed discussion of such systems is outside the scope of this review. However, the use of chemometric techniques for spectral compression and searching is described in some recent publications [178–182].

Fluorescence spectrometry

The book by Wehry gives a good introduction to some of the newer approaches to fluorescence spectrometry [183]. In contrast to absorption methods, fluorescence produces a 3-dimensional data matrix of emission intensity versus excitation wavelength versus emission wavelength. Multicomponent analysis on the matrix can be carried out by the least-squares method [184]. It is also possible to analyse known compounds in mixtures by rank annihilation factor analysis [185, 186].

Synchronous scanning is carried out by scanning both excitation and emission wavelengths simultaneously with fixed interval ($\Delta\lambda$) between the two wavelengths [187]. It is equivalent to taking a slice through the 3-dimensional data matrix at an angle of 45°, and can be used to reduce the width of emission peaks. The technique is capable of multicomponent analysis [188]. A recent variation is variable angle synchronous scanning, which can be used to improve specificity further and has been applied to chlorpromazine and tetracycline [189]. Derivative fluorescence spectrometry is also useful for reducing peak widths in the analysis of mixtures [190, 191].

Facilities such as derivative spectrometry and synchronous scanning are available on modern fluorimeter systems with data handling. The 3-dimensional data matrix can usually be obtained and converted to a contour plot which gives useful information on the numbers and shapes of peaks. Facilities for rank annihilation factor analysis are not generally available.

Commercial instrumentation is now available for phase modulation fluorescence, which discriminates between molecules on the basis of lifetimes. The technique has been used for quantitative analysis, but without sophisticated data treatments as yet [192, 193].

Other Analytical Techniques

A number of other analytical techniques are important in pharmaceutical analysis. They include titrimetry, flow injection analysis, atomic absorption and emission spectrometry, potentiometry and electrochemistry.

Titrimetry

For conventional titrimetry, with a single reacting species, it is sufficient to determine the end point by differentiating the output from the sensor (often a glass or other electrode). This technique is used in several commercial instruments. More sophisticated treatments are needed when determining pK_a particularly with polyfunctional molecules. An iterative procedure, which caters for errors in the measurements of both the volume and potential, has been described [194]. Mixtures may be analysed by titration, using computer analysis to estimate the pK_a and concentration of each component [195].

Linear titrations (e.g. with potentiometric or conductometric detection) give curves in which it can be difficult to estimate the end point. A statistical equation is available to estimate the standard deviation of the result [196]. The use of the Kalman filter in end point estimation has recently been investigated [197].

Flow injection analysis

Flow injection analysis may be used to automate many analyses, particularly involving visible spectrophotometry. It is also applicable to other spectrophotometric methods and to titrations. For optimisation of conditions, the modified simplex method has been shown to be better than univariate methods [198].

Atomic absorption and emission spectrometry

Atomic absorption and emission methods are often calibrated by standard addition, and the earlier references on calibration and the generalised standard addition method are relevant. Optimisation of parameters has been reported by the simplex method [199] and either by simplex or by modelling the response surface [200]. The precision may be improved by suitable experimental design [201]. Factorial analysis may be used to compensate for matrix interferences with both linear and non-linear responses [202].

Electrochemistry

Fused peaks in voltammetry may be quantified by determining the least-squares fit of standard spectra after carrying out a Fourier transform [203]. This approach allows a smoothing filter to be applied to the transformed data before the least-squares fit is carried out. The adaptive Kalman filter may be used to quantify mixtures where there is some interference from unknown components, as in spectrometry [69].

The Generalised Standard Addition Method may be used to compensate for non-ideal behaviour in anodic stripping voltammetry due to the formation of intermetallic compounds [204].

Conclusions

Chemometrics is having an increasing impact on pharmaceutical analysis and this impact will become even more profound in the next few years. The authors hope that this survey has indicated the current areas of application and that, as a result, analysts will be encouraged to read the work cited herein and will see the benefits that chemometrics can bring to their own work.

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