Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 4, pp. 329–336, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Automation of pharmaceutical dissolution testing by flow injection analysis

M.D. LUQUE DE CASTRO and M. VALCÁRCEL

Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, 14004 Córdoba, Spain

Abstract: The different types of instruments used for monitoring pharmaceutical dissolution testing are presented. Their features and the need for automation are critically discussed. The advantages of flow injection analysis in this respect are illustrated by a variety of examples clearly showing its adaptability to the different problems posed by other automatic and non-automatic alternatives.

Keywords: *Automation; flow injection analysis; dissolution testing.*

Introduction

Dissolution testing of drugs in tablets or capsules has become an essential routine procedure accounting for a substantial portion of analytical workload in the pharmaceutical industry. Although it may seem to be a matter of common sense that dosage forms should be disintegrated and dissolved before they are made active, the first proposal for a quantitative test was made in 1960 by Levy and Hayes [1], who compared buffered and unbuffered acetylsalicylic acid preparations. Since then, problems associated with the biological availability of drugs are being paid increasing attention by industry, government agencies and standard compendial. The matter was forcibly brought into focus by a routine investigation of identical competitive products marketed by prominent pharmaceutical manufacturers.

The principle behind dissolution tests involves the *in vitro* tracking of the significant events occurring when drugs are taken orally (and, more recently, transdermally). All of the methods developed for this purpose involve establishing a renewable solid–liquid interface between the dosage form and the dissolution fluid that can be defined and controlled and hence reproduced. Effective *in vitro* dissolution studies have, more often than not, been considered the stepping stones towards the production of therapeutically effective drug delivery systems. For long, researchers have been confronted with the challenge of devising an *in vitro* dissolution system closely mimicking the environment of a biological system. The many attempts made in this direction have been rewarded with comparable *in vitro* and *in vivo* results. Nevertheless, there is some controversy as regards the usefulness and validity of *in vitro* dissolution methods as far as the correlation with *in vivo* results is concerned. The development of an *in vitro* method allowing the *in vivo* performance of a specific drug to be predicted would be the first step towards developing usable dissolutions systems. Unquestionably, poor *in vitro/in vivo* correlations may in some instances reflect the variability of the *in vitro* dissolution procedure employed, as well as inter and intrasubject *in vivo* variation. No universal dissolution test method resulting in the same rank order for *in vitro* dissolution and *in vivo* availability for different formulations or batches in every instance has yet been devised [2]. There is an acknowledged scarcity of data about the correlation between the *in vitro* and *in vivo* performance of drugs and related products. However, the current interest and activity in this area indicates that such data are bound to be available shortly. With the increasing knowledge gathered, the pitfalls of *in vitro* methods are being exposed and refinements in equipment and procedures are being introduced. At present, the ever increasing complexity of formulations, lower doses and more sophisticated delivery systems used (timed release and retarded release dosing forms) call for new developments in this area to suit existing methodologies and instrumentation to the problems posed by the new generations of pharmaceuticals.

The latest developments in dissolution testing are being orientated in two directions namely:

(a) Improvements in dissolution testers

Despite the fact that official methods (USP basket [3, 4] and USP paddle [4] apparatus) dramatically curtail the possibility of using newer, more interesting instruments, a number of improved procedures have been reported (tumbling [5], beaker [6], rotating disc [7–10] methods, non-automatic [11–14] and automatic [15] modifications of the USP basket method, continuous flow systems [16–19], controlled pressure apparatus [20]);

(b) Improvements in the performance of dissolution process monitoring

These are more affordable as they are subject to no restrictions from Pharmacopeiae. In addition, the ever increasing complexity of drug delivery systems are making conventional UV assays inadequate [21]. The different alternatives devised in this context are commented on below and their advantages and disadvantages are critically discussed, placing special emphasis on flow injection analysis [22, 23].

Alternatives to Dissolution Testing Monitoring

In addition to the new, more complex dosage forms available today, the growing number of controls required call for the automation of dissolution testing monitoring. The different possibilities reported in this context and the various degrees of automation with which they can be implemented are discussed below.

Figure 1 shows the different ways in which dissolution processes can be monitored.

By conventional aspiration systems

The continuous flow aspirated from six vessels by a rotating basket or paddle (Fig. 2) can be monitored in two ways depending on the features of the instrument: (i) by using an automated selecting valve sequentially driving a small volume of the evolving system to a single flow-cell with usually a photometric detector; (ii) by using a dedicated detector furnished with six flow-cells, each of which receives the stream from one vessel. Both approaches involve monitoring one of the native features (usually the absorbance) of an active compound. The instruments manufactured by Beckman and Philips are based on this principle. In both cases, the six flow-cells used allow the content of the six vessels to be monitored simultaneously or sequentially (in the latter case by moving the sample compartment sequentially to different positions along the spectrometer light path).

Although these approaches provide near-real time response, they are unusable with derivatizing reactions, on-line separation processes, etc. when the analyte has no physicochemical properties for direct monitoring, or when some interferents are present in the dosage form. The return of the flow to the corresponding vessel is mandatory to keep the dissolution conditions unchanged.

With a sampling interface

The use of a sampler located between the dissolution vessel and the instrument allows a number of approaches to be developed based on the particular instrument used. Spectrometers are the most commonly used detectors, though there is a current unjustified trend to using chromatographs even when no separation or pre-concentration is required.

A custom-made fast liquid chromatographic system was reported by Bonald *et al.* [24]. This

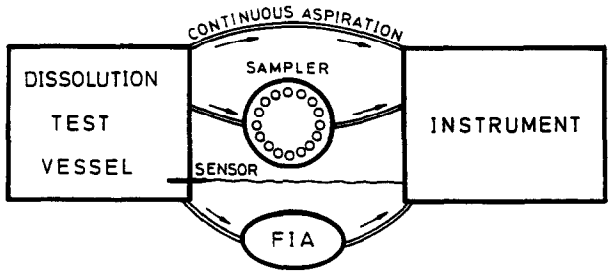

Figure 1
Different ways of monitoring the dissolution processes.

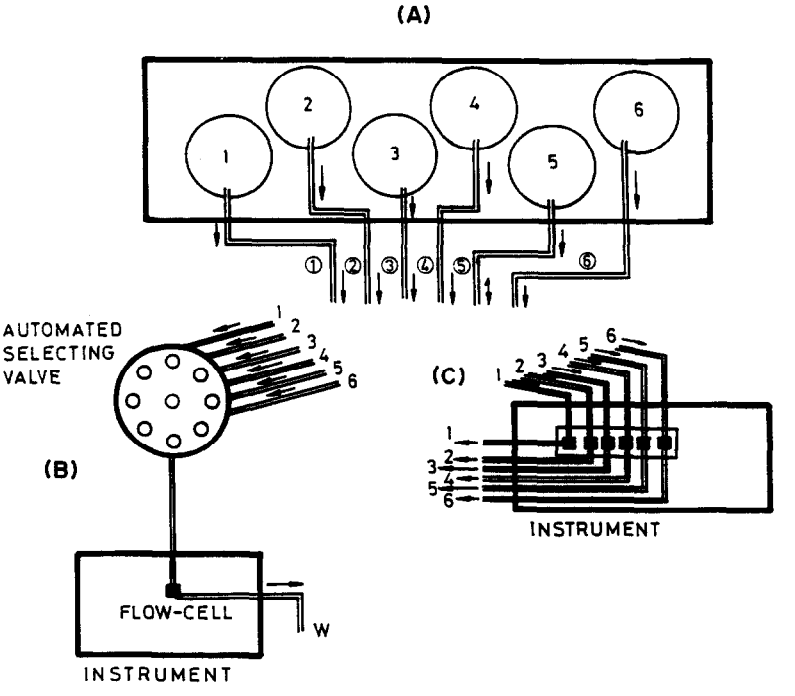

Figure 2
Conventional ways of monitoring the dissolution processes occurring simultaneously in six vessels: (A) as established by the USP; (B) with automated selecting valve positioned prior to a detector with a single flow-cell; (C) dedicated instrument with one cell per stream (or vessel).

consisted of a peristaltic pump for dissolution sampling, a commercially available HPLC autosampler equipped with specially designed injection circulating vials and other standard equipment required. The fast HPLC technique allows six samples to be analysed between two successive dissolution sampling times throughout the dissolution test simultaneously conducted in six vessels. The wide applicability of the system was illustrated with two drugs: (a) a new low UV-absorbant (λ_{max} = 200–210 nm) chemical entity formulated in capsules, the dissolution of which was complete within 45 min, and (b) a commercially available antiarrhythmic compound in tablets with fast dissolution kinetics. The sampling interval was 7.5 min.

A complete instrument assembly from Waters includes a dissolution bath and transfer control, a special sample processor, HPLC column, pump, detectors and data system. For economy, well-proved subassemblies are used, but their functions are coordinated and governed by a data and chromatographic control station and a computer, which is actually a decision-maker. Depending on the dissolution sampling interval chosen, it allows the completion of analyses, collects samples and serially analyses them, or decides, for example, to inject only three replicates for analysis before proceeding with the next sampling and delaying the work-up of the three remaining samples, which are then run in an open-time slot [25].

The chief drawbacks of the use of a sampler as an interface are the impossibility of returning the excess sample to its vessel and the far from real-time response achieved.

When the dissolution process is lengthy and samplings must be made relatively frequently, the overall aspirated volume should be controlled in order to keep the conditions of the dissolution process as constant as possible. Several approaches have been proposed to circumvent this problem. The Gilson autosampler [26] offers a compact apparatus with three-axis motion which can sample, dispense, pipet, dilute, mix and transfer liquids (Fig. 3). The work cycle develops in four steps: (i) liquid transfer from the test station into the receiving flask; (ii) dispensing of a preset volume of this liquid into a small vial; (iii)

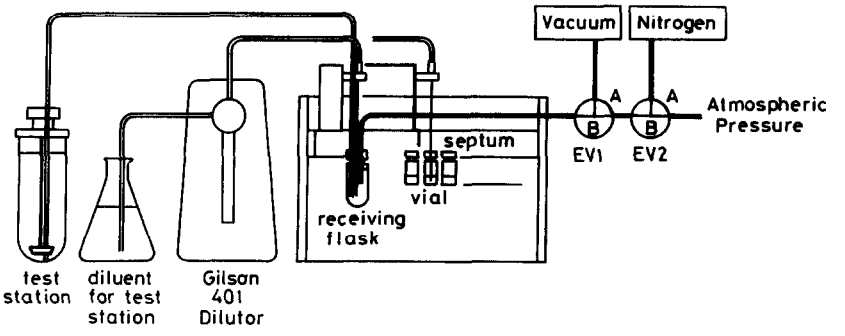

Figure 3
Diagram of the automatic sample collector manufactured by Gilson (for details, see text).

replenishment of the aspirated volume with an equivalent volume of diluent; (iv) transfer of the flask content to the test station.

The other major problem arising from the use of a sampler, i.e. the far from real-time response obtained, is important [27–29], especially when the active compound to be monitored, or some other component of the dosage form, is unstable.

By in-line monitoring

The monitoring of dissolution without sampling is desirable since most of the units otherwise required (sampler, aspiration system, etc.) become redundant. Approaches are, however, still at an early stage of development. Problems are posed by suspended particles usually present in the dissolution vessel making measurements difficult and/or fouling the sensor. Josefson *et al.* [30] proposed the use of a probe connected to a spectrometer via optical fibres to measure concentrations in opaque solutions without previous filtering of the sample. Measurements were made over a range rather than at a single wavelength and the statistical disturbance least-squares (PLS) method was used to correct for turbidity. This required running a calibration graph where a set of spectra from turbid samples was paired with single-wavelength measurements on the same samples manually filtered. The procedure, applied to tablet dissolution testing, featured reasonably small errors compared with the variation between individual tablets. The increased information from spectral rather than single-wavelength measurements also allows the quality of measurement to be monitored through residual and scatter plots. The authors proposed automation by robot.

By using a laboratory minirobot

The use of a robotic station is a usually convenient means of automating the various approaches to dissolution testing monitoring since manual methods can be converted to robotic without further optimization [31]. Good results have been obtained [32, 33]. Robotic stations can run up to 12 sets for six tablets, each with no human participation. The robot adds the dissolution test medium to the standard testing apparatus, introduces tablets at preset time intervals, arranges for measurement and calculates results. It then prepares the test vessels for the next assay by emptying, washing and refilling with test medium. Each vessel is checked for cleanliness before cost savings and reduction of the time required for gathering data. By running overnight, the robot can perform in 24 h the equivalent of a human working a 40-h week [34, 35]. A recently reported robotic approach [36] overcomes the two most difficult steps for conventional laboratory automation: filtration through a fine porosity membrane and HPLC end analysis. Two robotic arms combined into a unique system have been used to overcome these obstacles. The operation of one is based on an earlier custom-made robotic system with a membrane filtration module operating with either an UV or HPLC end analysis. The two systems communicate through power and event controllers. The total system has been extensively used and is capable of handling a variety of products providing reliable data in an efficient manner.

The most serious shortcomings of robotic stations are their high purchase and maintenance costs. When much versatility is required suitable software for each new application to be developed dramatically raises maintenance costs.

Flow Injection Analysis as an Advantageous Automated Aid to Dissolution Testing Monitoring

Flow Injection Analysis (FIA) makes an excellent interface between vessels and instruments as it solves the main problems posed by the systems considered above. Thus:

(a) it facilitates the use of derivatization reactions, thereby increasing selectivity and sensitivity and overcoming the drawbacks of continuous aspiration procedures;
(b) it allows the use of successive filters of different porosity prior to the detection system.

In addition, the use of FIA in dissolution testing monitoring is aimed at:

(i) offering a simple, low cost system featuring a higher degree of automation with greater potential than currently available commercial instrumentation;
(ii) affording the monitoring of rapidly evolving systems involving active compounds (near-real time responses);
(iii) meeting the need for the monitoring of systems involving very fast dissolution kinetics (high sampling rates);
(iv) on-line operation, and FIA advances in multideterminations allow the monitoring not only of the active compound but also of its potential degradation products;
(v) affording the monitoring of systems used for compounds with extremely slow dissolution kinetics or for their degradation products. This requires continuous on-line preconcentration;
(vi) diversifying the detection modes used to date with dissolution tests in order to increase the potential of mono and multi-determination;
(vii) expanding the linear range of determination, thus avoiding preconcentration or prior dilution [37, 38].

The earliest attempt at using FIA to monitor dissolution tests was made by Koupparis *et al.* [39], who used control by microcomputer software. A calibration graph was first obtained using standards of the compound examined (phenothiazine). A dosage form was then placed into a screen basket which was kept spinning in a double wall beaker shown in Fig. 5(A). The dissolution medium was recirculated continuously through the sample loop and was injected into a carrier stream at preset time intervals. The results obtained, namely the entire dissolution profile on the chart recording (a series of absorbance versus time peaks) are shown in Fig. 5(B). Another, more recent contribution, of this group was the determination of sulphonamides in tablets by using the Bratton–Marshall reaction [40].

A straightforward two-channel FIA manifold was used to show the feasibility of this technique to monitor ascorbic acid tablets with

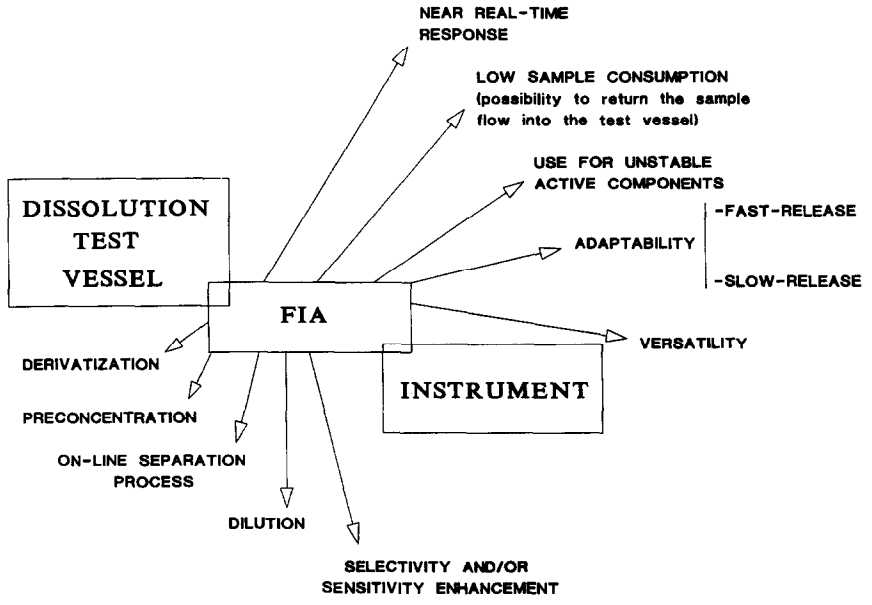

Figure 4
Advantages of the use of FIA as an interface between dissolution testing vessels and instruments.

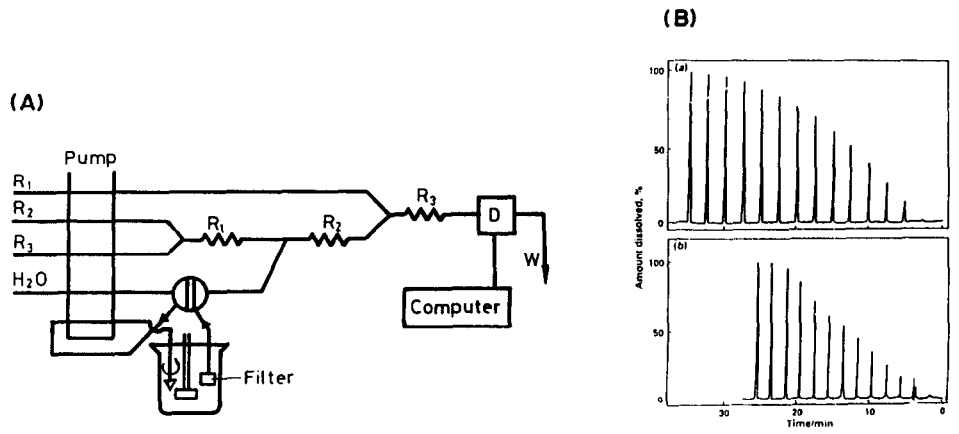

Figure 5
(A) FIA manifold for the monitoring of dissolution of tablets containing phenothiazine. (B) Chart recording of the overall dissolution process.

very different dissolution kinetics [41]; Redoxon^R tablets (very fast dissolution rate) and timed-release Treasury^R tablets furnished with a special molecular sieve which retarded the dissolution process, were used for this purpose. The chloramine-T method provided a sufficiently fast derivatizing reaction. The sampling frequency achieved was 90 h^{-1}, i.e. more than adequate for both kinetic rates.

The coupling of FIA and separation techniques as interface between vessels and detection instruments further enhances the possibilities of FIA systems. A separation technique can be readily coupled on-line with an FIA manifold to provide the required level of selectivity (elimination of interferents) or sensitivity (preconcentration step). There are several examples in the FIA literature showing the feasibility of this approach. Thus, Lamparter and Lunkenheimer developed methods for (i) the determination of content uniformity of Aliniding tablets using an automated ion-pair extraction method; (ii) for the determination of dissolution rates of Brotizolam in Lendormin tablets and of WEB 2036 Bs in gelatine capsules using fluorimetric detection and (iii) a new type of dialysis cell to separate the excipients from the active ingredients [42]. More recently, Nord *et al.* [43] developed an automatic FIA-extraction method for the monitoring of the dissolution of felodipine tablets. The water-soluble oxidation product of the analyte, a pyridine derivative, was extracted into chloroform and measured spectrometrically at 275 nm. The FIA-extraction method was compared with the reference liquid chromatographic (LC) method. The sampling rate for the FIA-extraction method (60 samples/h) was 5 times higher than for the LC method. Both had a relative standard deviation of 1% for standards and samples.

The automated system depicted in the block diagram in Fig. 6 was patented by Valcárcel and Luque de Castro [44]. It is a highly versatile modular design adaptable to a variety of needs and consists of four modules: (i) a dissolution unit comprising three or six externally thermostatically controlled glass vessels with individual stirring systems (paddle or basket); (ii) a storage/solvent addition/waste/washing unit consisting of one or several solvent reservoirs connected through a distributing valve to each of the dissolution vessels in the dissolution unit. This device allows the vessel content to be discarded and the vessel washed and refilled for automatic reuse; (iii) an FIA analyser suited to the particular requirements and consisting of one or several peristaltic pumps, injection and selecting valves, reactors, a separation system if needed and an optical or electroanalytical detector; (4) a microcomputer furnished with an active interface to control the above three modules and a passive interface to collect signals from the system and deliver them directly through the printer as required.

Conclusions

The needs of *in vitro* dissolution testing call for the development of faster, more versatile automated instrumentation to meet present demands and the application of FIA has many advantages such as adaptability to different

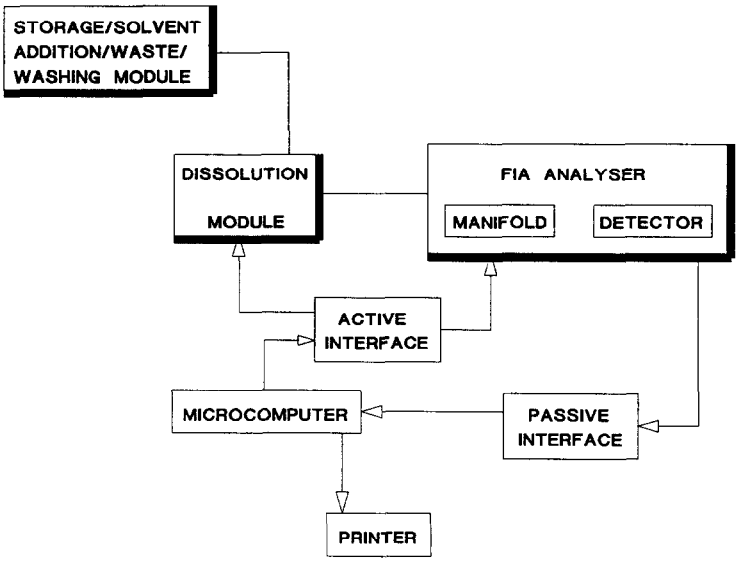

Figure 6
Fully automated modular system for dissolution testing and monitoring, featuring a versatile flow injection manifold acting as an interface between the dissolution vessel and the instrument.

requirements including varying degrees of automation, on-line derivatizing reactions, separation techniques and different types of detection. It has valuable intrinsic features including near-real time response, low sample and reagent consumption, inexpensive components and easy manipulation.

References

[1] G. Levy and B.A. Hayes, *New Eng. J. Med.* **262**, 1053–1066 (1960).
[2] U.W. Banakar and L.H. Block, *Pharm. Technol.* **7**, 107–112 (1983).
[3] A. Richter, B. Myhre and S.C. Khanna, *J. Pharm. Pharmacol.* **21(Suppl)**, 409 (1969).
[4] D.C. Cox, C.C. Douglas, W.B. Furman, R.D. Kirchhoefer, J.W. Myrick and C.E. Wells, *Pharm. Technol.* **2**, 41–50 (1978).
[5] M.S. Wruble, *Am. J. Pharm.* **102**, 368–378 (1930).
[6] I.C. Edmundson and K.A. Less, *J. Pharm. Pharmacol.* **17**, 193–206 (1965).
[7] G. Levy and B.A. Sahli, *J. Pharm. Sci.* **51**, 58–66 (1962).
[8] J.W. Wood, J.E. Syarto and H. Letterman, *J. Pharm. Sci.*, **54**, 1068–1075 (1965).
[9] W.E. Hamlin, E. Nelson, B.E. Ballard and J.G. Wagner, *J. Pharm. Sci.* **51**, 432–441 (1962).
[10] G. Levy and W. Tsanki, Jr, *J. Pharm. Sci.* **53**, 679–688 (1964).
[11] G. Haringer, B.J. Poulsen and R.N. Havemeyer, *J. Pharm. Sci.* **62**, 130–142 (1973).
[12] A.C. Shah, C.B. Peot and J.F. Ochs, *J. Pharm. Sci.* **62**, 671–682 (1973).
[13] A.C. Shah and J.F. Ochs, *J. Pharm. Sci.* **63**, 110–121 (1974).
[14] R.E. Shepherd, J.C. Price and L.A. Luzzi, *J. Pharm. Sci.* **61**, 1152–1164 (1972).
[15] E. Von Lamparter, H.J. Diederich, H. Eppelmann, K.O. Linn and H. Peil, *Pharm. Ind.* **49**, 621–626 (1987).
[16] M. Pernarowski, W. Woo and R.O. Searl, *J. Pharm. Sci.* **57**, 1419–1424 (1968).
[17] D.C. Baum and G.C. Walker, *J. Pharm. Sci.* **58**, 611–618 (1969).
[18] J.E. Tingstad and S. Riegelman, *J. Pharm. Sci.* **59**, 692–699 (1970).
[19] C. Cakirylildiz, P.J. Mehta, W. Rahmen and D. Schoenleber, *J. Pharm. Sci.* **64**, 1693–1702 (1975).
[20] S.S. Nasir, L.O. Wilken, Jr and S.M. Nasir, *J. Pharm. Sci.* **68**, 177–188 (1979).
[21] M.S. Wruble, *Am. J. Pharm.* **102**, 368–382 (1930).
[22] M. Valcárcel and M.D. Luque de Castro, *Flow Injection Analysis: Principles and Applications*. Ellis Horwood, Chichester (1987).
[23] J. Ruzicka and E.H. Hansen, *Flow Injection Analysis*. Wiley, New York (1988).
[24] M. Bonald, J.M. Arnouil, C. Magnet and R. Gimet, Int. Symp. on Pharm. Biomed. Anal., Barcelona, Spain (1987).
[25] I. Neitring nd E. Yapchanyk, *Am. Lab.* Nov. 62–67 (1987).
[26] C. Druon, *Int. Lab.* April 96–98 (1986).
[27] J.B. Callis, D.L. Illman and B.R. Kowalski, *Anal. Chem.* **59A**, 624–637 (1987).
[28] M.D. Luque de Castro, *Talanta* **36**, 591–599 (1989).
[29] W.E. van der Linden, G. de Niet and M. Bos, *Anal. Chim. Acta* **216**, 307–319 (1989).
[30] M. Josefson, E. Johansson and A. Torstensson, *Anal. Chem.* **60**, 2666–2671 (1988).
[31] M. Valcárcel and M.D. Luque de Castro, *Automatic Methods of Analysis*. Elsevier, Amsterdam (1988).
[32] A.N. Papas, M.Y. Alpert, S.M. Marchese and J.W. Fitzgerald, *Anal. Chem.* **57**, 1408–1411 (1985).
[33] R.M. Sample, *Anal. Proc.* **23**, 266–267 (1986).
[34] L.J. Kostek, B.A. Brown and J.E. Curley, *Zymark Pub.* 701–720 (1985).
[35] R. Wolfschutz, H. Ganshirt and H.G. Tessun, *Zymark Pub.* 139–152 (1986).

[36] L.J. Kosdtek, B.A. Brown, L.C. Erhart and J.E. Curley, *Zymark Pub.* 311–328 (1988).
[37] A. Ríos, F. Lázaro, M.D. Luque de Castro and M. Valcárcel, *Anal. Chim. Acta* **199**, 15–27 (1987).
[38] J.M. Fernández-Romero, M.D. Luque de Castro and M. Valcárcel, *Anal. Chim. Acta* **219**, 191–199 (1989).
[39] M.A. Koupparis and A. Barcuchova, *Analyst* **111**, 313–318 (1986).
[40] M.A. Koupparis and P.I. Anagnostopulou, *Anal. Chim. Acta* **204**, 271–283 (1988).
[41] J.S. Cosano, A. Izquierdo, M.D. Luque de Castro and M. Valcárcel, *Pharm.* in press.
[42] E. Lamparter and Ch. Lunkenheimer, *Pharmazie* **3**, 215–219 (1988).
[43] L. Nord, M. Sundgren and A. Torstensson, *J. Pharm. Biomed. Anal.* **7**, 421–431 (1989).
[44] M. Valcárcel and M.D. Luque de Castro, Spanish Patent No. 8703795 (1987).

[*Received for review 11 December 1989*]