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ADVANTAGES OF THE USE OF VERY SHORT AND ULTRA SHORT HPLC COLUMNS FOR DRUG ANALYSIS IN DISSOLUTION TESTING

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ABSTRACT

The advantages of short (3-5cm long) or ultra short (4mm long) HPLC columns for drug analysis in dissolution testing are illustrated by reference to some example antihypertensive drug formulations.

Advantages include:

selectivity, where interfering excipients or co-formulated drugs complicate UV spectrophotometric analysis:

time savings, compared with conventional HPLC columns (although where UV spectrophotometry is applicable no time advantages may be obtained with short column HPLC):

economy, as the columns are less expensive than conventional columns and there is reduced solvent consumption:

increased sensitivity compared with conventional columns. Low dose potent drugs with poor chromophores may be more readily quantitated:

amenity to automation, including the use of laboratory robots.

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These advantages would suggest the wide applicability to this mode of analysis in dissolution testing.

INTRODUCTION

In-vitro dissolution testing is an important tool in the development and quality control of solid dose forms. Basically, the test consists of measuring the rate of release of drug from a solid dose form into an aqueous environment under defined conditions. Samples of test solution are taken at one or more time points and the concentrations of dissolved drug or drugs are determined. In a typical test six tablets or capsules would be tested simultaneously.

Traditionally, UV spectrophotometry has been used to determine the amount of dissolved drug. However, modern potent drugs may be present in low doses and additionally have weak chromophores, making spectrophotometery difficult. A number of drugs may be co-formulated into a single dose unit to aid patient compliance. Such formulations may present problems in spectrophotometric analysis requiring measurement at multiple wavelengths with associated calculations, or the dedication of an expensive photodiode array spectrophotometer.

High peformance liquid chromatography (HPLC) may provide an answer in selectivity and sensitivity to resolve the above-mentioned problems, but can be slow, with each sample analysis taking up to thirty minutes to complete. Such analysis times would mean that samples need to be deposited in a fraction collector for subsequent analysis, although Wurster et al (1) have already described an automated dissolution system with direct introduction of samples into an HPLC system. This latter system is only applicable to analysis of samples

from a single dissolution vessel in most instances. With conventional HPLC columns, analysis times are such that direct sampling from a number of vessels at time points separated by only a few minutes would not be possible. To obtain the benefits of HPLC and reduce analysis time, we have applied very short (3-5cm. long) HPLC columns and 4mm long guard columns as ultra short analytical columns to the analysis of drug solutions obtained during dissolution testing.

There are some published reports on the use of very short columns in pharmaceutical analysis (2,3), but the only publication on the application to dissolution testing (3) highlights the speed of such techniques and their amenity to automation.

The present work demonstrates the advantages of short column HPLC when applied to some example antihypertensive formulations, where potent drugs with weak chromphores or combination formulations are involved. The further advantages and general applicability of this approach to drug analysis in dissolution testing are highlighted.

EXPERIMENTAL

Equipment

Dissolution tests were undertaken in a USP rotating basket apparatus having six test stations (Hanson 72 RL, Copley Instruments, Nottingham, U.K., or Caleva, DT7, G.B. Caleva, Ascot, U.K.). Samples were taken manually into 10ml. plastic syringes or automatically employing an autosampler (Caleva 3-10, G.B. Caleva, Ascot U.K.). Filtration was achieved using disposable filters (Gelman 25mm Acrodisc, 0.45μ pore size, Gelman Sciences, Northampton, U.K., and porous polypropylene in-line

filters, cat. no. 1504, Copley Instruments, Nottingham, U.K., respectively).

Chromatography was undertaken employing a constant volume reciprocating pump (Altex 110A, Anachem, Luton, U.K.), a variable wavelength detector (Cecil CE212A, Talbot Instruments, Alderley Edge, U.K.), and an autoinjector (Talbot AS13, Talbot Instruments, Alderley Edge, U.K.). Chromatograms were recorded on a Servosorbe 1S chart recorder (Brunner Instruments, Scarborough, U.K.).

Very short HPLC columns were packed with 3μ C18 reversed-phase material (Perkin Elmer, Beaconsfield, U.K. or Technicol, Stockport, U.K.). Ultra short columns were 4mm. long C18 reversed phase guard column cartridges held in a guard column holder (Waters Instruments, Harrow, U.K.), and were employed in place of the conventional analytical column.

Applications

Tablets containing 12.5mg. of the antihypertensive agent captopril (E.R. Squibb, Moreton, U.K.) were subjected to a dissolution test employing 1000ml. of 0.1M hydrochloric acid in each vessel as dissolution medium. Chromatographic analysis was undertaken on a 3cm. long C18 reversed phase column and an eluting solvent consisting of methanol:water:85% phosphoric acid (380:620:0.4 v/v/v) was employed at a flow rate of 1.0ml. min^{-1}. The detector was set at 218nm. and 50μl of sample injected via the autoinjector.

A development antihypertensive agent formulated as 10mg. of drug in capsules (E.R. Squibb, Moreton U.K.) was subjected to a dissolution test employing 500ml. of 0.1M hydrochloric acid as dissolution medium. Chromatographic analysis was undertaken on a 4mm. long C18 reversed phase guard column used as an ultra short analytical column. The solvent was methanol:phosphate buffer (pH 2.0) (3:7

v/v) delivered at 0.2ml. min^{-1}. The detector was set at 221nm. and 50µl of sample injected.

A combination formulation containing 50mg. of captopril and 15mg. of the diuretic hydrochlorothiazide in a tablet (E.R. Squibb, Moreton, U.K.) was subjected to dissolution testing using the rotating basket method and employing 1000ml. of 0.1M hydrochloric acid as dissolution medium. For simultaneous analysis of both drugs, HPLC on a 5cm. long reversed phase C18 column was employed, using methanol:0.5% aqueous phosphoric acid (20:80 v/v) at 1.0ml. min^{-1} as eluting solvent. Injection volume was 20µl and the variable wavelength detector was set at 210nm. to obtain suitable peak height relationships, as the two drugs involved have very different chromophores.

RESULTS AND DISCUSSION

In-vitro dissolution testing of the antihypertensive drug captopril involves Ellmans reagent colorimetry of the thiol group for drug analysis. However, this approach requires preparation of a number of reagents, colour development and finally spectrophotometry. Hence the method is time consuming, although automation of such methods is possible. Furthermore, the method is non-specific, as other thiol compounds, for example captopril hydrolysis products (4), would react with the reagent.

A specific HPLC method used in preformulation studies on captopril (4) can be used to separate and quantitate captopril in dissolution media. By use of a 3cm. long column, rather than a 20cm. long column, total analysis time per time point sample was reduced to less than two minutes. A typical chromatogram is shown in Figure 1. Compared with the 20cm. long column the time to complete analysis of four time point samples from each

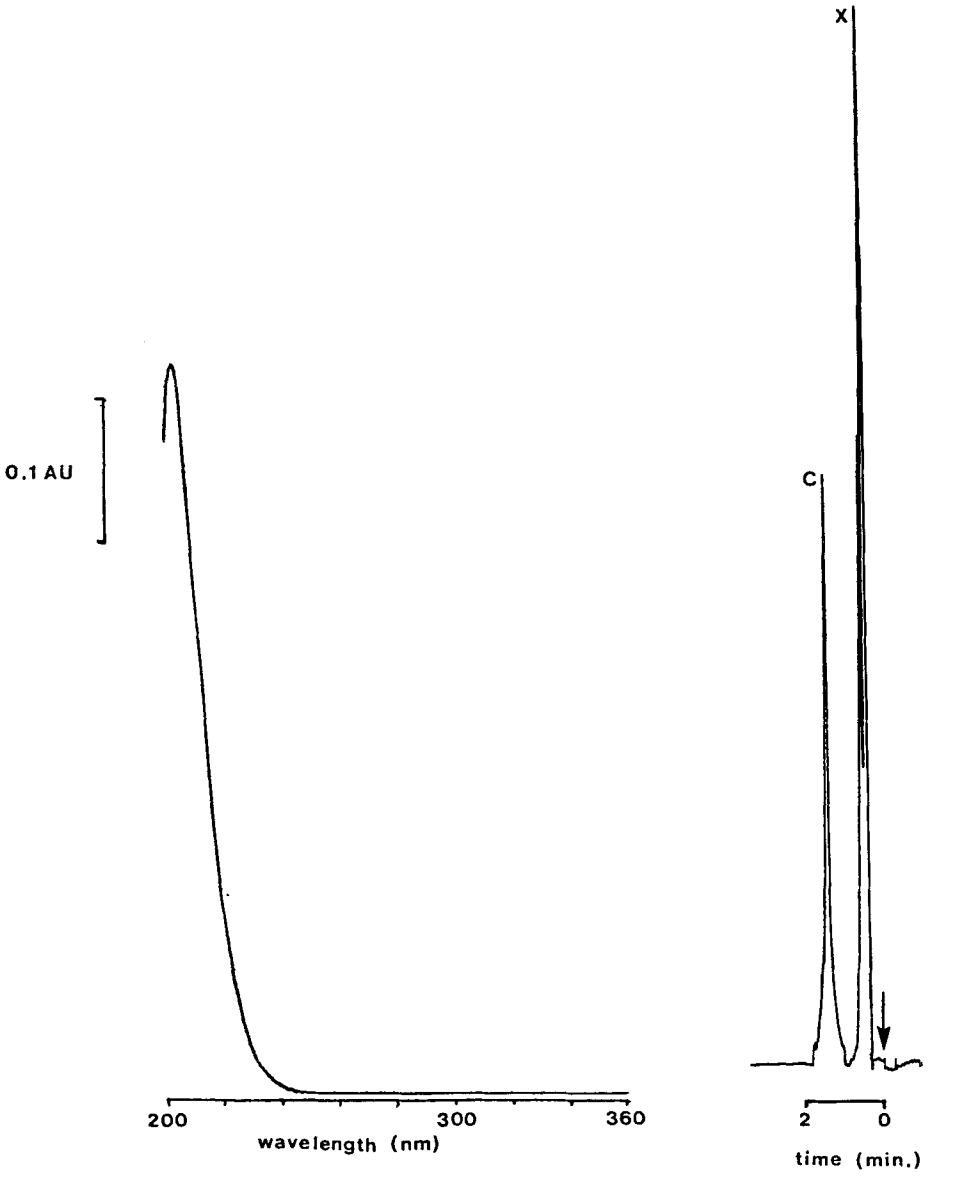

FIGURE 1

(left) UV absorption spectrum of 12.5mg captopril dissolved in 1000 ml. of 0.1N hydrochloric acid.
(right) Chromatogram of dissolution medium during dissolution test on 12.5mg captopril tablet.
C = Captopril; X = excipients.

TABLE 1

Times to complete determination of captopril concentrate for four time point samples from each of six vessels during dissolution testing

	Colorimetry	HPLC (20cm.col.)	HPLC (30cm.col.)
Reagent or HPLC solvent preparation	2.5hrs.	0.3hrs.	0.3hrs.
Colour Development	0.3hrs.	-	-
Analysis	0.3hrs.	4.2hrs.	0.9hrs.

of six dissolution vessels was reduced from around four hours to less than one hour. Table 1 indicates the total times required to complete analysis of four time points from each of six dissolution vessels by colorimetric and HPLC methods.

Because of the time savings, HPLC solvent consumption was also reduced. The shorter column length resulted in an approximately five fold increase in sensitivity. This latter advantage allowed for the quantitation of captopril from a 12.5mg. potency tablet formulation in 1000ml. of dissolution medium, in spite of the weak end absorption chromophore of captopril (Fig.1).

With a second development antihypertensive agent separation of the active drug from capsule excipients could be achieved on a 4mm. long guard column (Fig. 2), which could be regarded as an ultra short analytical column. In this case analysis is very rapid providing significant time savings, reduced solvent consumption and inexpensive columns when replacements are required. Such rapid analyses are suitable for on-line use, with a

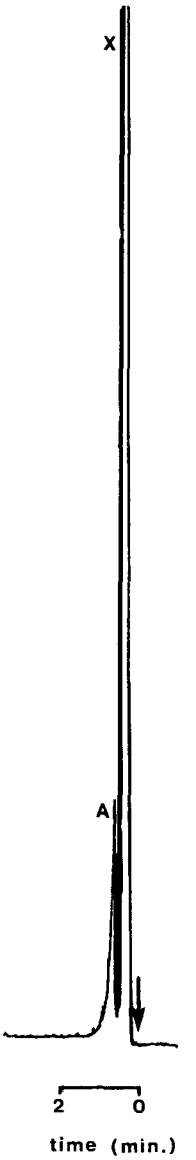

FIGURE 2

Chromatogram of dissolution medium during dissolution test on a development antihypertensive agent (A) using an ultra short column.
X = excipients.

laboratory robot or a sampling valve sequentially selecting from each dissolution vessel and a standard solution. Time between cycles would be short enough to permit dissolution profiling of even rapidly dissolving formulations in this way.

Combinations of the diuretic hydrochlorothiazide with captopril could be analysed in dissolution media by HPLC. Separation was on a very short reversed phase C18 column allowing simultaneous quantitation, saving considerable time over traditional individual colorimetric methods. With the colorimetric methods it may only be possible to analyse one or two batches of tablets manually,per day, whereas the HPLC method could theoretically allow for analysis of four or more lots. A typical chromatogram for the separation of captopril and hydrochlorothiazide in dissolution media on a 5cm. long column is shown in Figure 3. The very short column methods provide for savings in time, solvent and expense compared with typical 25-30cm. columns previously used to analyse these components in intact tablets (5) and that are applicable to dissolution testing.

There are some problems with the use of HPLC to analyse samples of dissolution media. Acidic media such as 0.1N hydrochloric acid can reduce column lifetime to just a few weeks. Fortunately such columns are less expensive than their conventional counterparts. In the examples given here a relatively inexpensive detector has been satisfactory but for very rapid analysis it is desirable to use a more expensive detector with a short time constant to obtain optimal resolution of closely eluting peaks (2).

Finally, although general applicability of this method is possible, the application to simple formulations where direct spectrophotometry is straightforward (e.g. high dose drugs with good

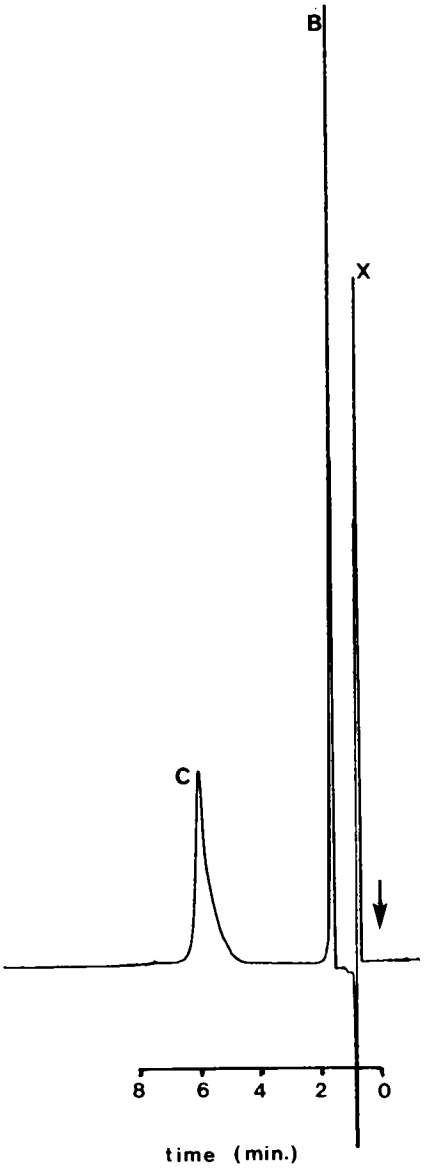

FIGURE 3

Chromatogram of dissolution medium during dissolution test on a tablet containing captopril and hydrochlorothiazide. C = Captopril;
B = hydrochlorothiazide;
X = excipients.

chromophores), may lead to no advantages and tie up expensive HPLC equipment.

In summary, the advantage of very short or ultra short HPLC columns in dissolution testing are-

Selectivity, allowing separation of co-formulated drugs for simultaneous quantitation. Where reduced selectivity is acceptable, guard columns may be used as ultra short columns to separate drug from excipients.

Time saving, as reduced analysis times compared with conventional HPLC columns are obtained.

Economy as columns are less expensive than conventional columns and reduced analysis times lead to reduced solvent consumption.

Sensitivity is increased due to the reduced column length, hence low dose potent drugs with weak chromophores may be more readily quantitated compared with conventional columns.

Amenable to automation. With the very short analysis times it is feasible that dissolution medium can be sampled directly into the chromatograph, either by selection and injection valves or via laboratory robots.

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