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Use of ultra-performance liquid chromatography in pharmaceutical development

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Abstract

Ultra-performance liquid chromatography (UPLC) has been investigated as an alternative to HPLC for the analysis of pharmaceutical development compounds. We present data on three compounds showing that significant reductions in separation time can be achieved without compromising the separation quality. Results from precision and comparative studies indicate that UPLC is a suitable technique for routine pharmaceutical analysis.
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1. Introduction

1.1. The role of HPLC in pharmaceutical development

Pharmaceutical development is an important part of the phase in between the identification of a chemical entity with therapeutic potential and the launch and routine use of a new medicine. A number of activities are involved including the scale up of the synthetic route from bench to plant scale, and the development of a tablet or other dosage form of the new medicine which can be manufactured at a large scale. Analytical chemistry plays an important role in supporting these activities by helping to understand the impact of changes in the route and scale of manufacture on the quality and consistency of the dosage form.

HPLC is one of the main analytical techniques used in controlling the quality and consistency of both the chemical entity, or active substance, and the dosage form. For example, HPLC is used in determining the purity of different batches of the chemical entity and so helps to ensure that material used in clinical trials is of a similar quality to that which has been assessed in toxicological studies. HPLC is also used to determine whether any degradation of the chemical occurs within the dosage form over time and so helps to establish the shelf life.

1.2. Improving efficiency in HPLC

Whilst HPLC is a very well established reliable technique, and is adequate in controlling pharmaceutical quality and consistency, it could still be improved. For example, one problem is that HPLC is often a slow technique because of the complexity of some of the pharmaceutical samples encountered. For example, samples may contain several impurities at levels of around 0.1% relative to the active substance. Such a concentration range means that the separating column must have high sample capacity in addition to high efficiency. These low level impurities can include species such as residual intermediates, analogues of the active substance, isomers, and degradation products. These must be separated sufficiently from the active substance and from each other so that their concentrations can be reliably measured. Because of the range of polarities involved gradient separation are often required for purity assessment, and because of sample complexity separation times of 30 min or more are not uncommon. Reducing these separation times without reducing the quality of the separation would mean that important analytical information could be generated more quickly.

Reducing separation times in HPLC without reducing the quality of the separation requires generating higher resolving power per unit time. Whilst the resolution between individual analytes in a particular sample may be increased by improving selectivity or retention, the best general approach to increasing resolving power is to increase separating efficiency.

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In this paper the use of a smaller diameter packing and higher operating pressures has been explored as a way of generating higher separating efficiencies. A commercial system capable of generating much higher pressures (1000 bar) than used in standard HPLC has been evaluated to determine its potential in routine analysis.

1.3. The need for higher pressures

Efficiency in HPLC is a function of operating conditions. Extensive experience has shown that the height of the column equivalent to a theoretical plate (HETP) varies with the mobile phase linear velocity. Efficiency in packed column HPLC can be described by the plate height model used in the van Deemter equation, and other equations of a similar form. In this work, Eq. (1) has been used as it makes explicit the impact of column properties, analyte properties and operating conditions on column efficiency [1].

$$H = Ad_{\mathrm{p}} + \frac{BD_{\mathrm{M}}}{u} + \frac{Cd_{\mathrm{p}}^{2}u}{D_{\mathrm{M}}} \quad (1)$$

where H is the HETP, d_{p} the particle size of the column packing material, u the linear velocity of the mobile phase, D_{M} the analyte diffusion coefficient and A–C are the constants. A relates to the fact that the flow path through a packed bed is tortuous and so not all analyte molecules have paths through the column of equal length. B relates to molecular diffusion (in the direction of the column axis). C describes the mass transfer of the analyte in the mobile and stationary phases. [1].

Other equations of greater complexity than 1 can be employed depending on the purpose. For a fuller analysis, description, and discussion of the underlying physical processes that underpin chromatography the reader is referred to more specialised texts [1,2].

Eq. (1) shows that efficiency varies with linear velocity, and the nature of the second and third terms of the equation indicates a minimum value for HETP. This minimum occurs at linear velocities that are much lower than those typically employed with stationary phase particles in the range of 5–3 μm in diameter. In the third term of Eq. (1), the particle size is squared and so the curve is steeper for larger particles at high linear velocities. This means that in order to reduce analysis times to acceptable values columns packed with common particle sizes are often operated at linear velocities which do not give maximum efficiency.

The position of the minimum on the HETP curve, and the optimum linear velocity, can be determined by the use of differential calculus [2]. The optimum linear velocity occurs when the slope of the H versus u curve is zero, i.e. when $\mathrm{d}H/\mathrm{d}u = 0$. This condition is satisfied when:

$$u_{\mathrm{opt}} = \frac{D_{\mathrm{M}}}{d_{\mathrm{p}}}\sqrt{\frac{B}{C}} \quad (2)$$

Eq. (2) shows that the optimum linear velocity is inversely related to the particle size, and directly proportionate to the analyte diffusion coefficient. Optimum operating conditions depend upon the analyte as well as the column employed. The use of smaller diameter particles should allow the use of higher linear velocities and so shorter analysis times. Smaller analytes (larger diffusion coefficients) can be analysed at higher linear velocities.

The value of H at the optimum linear velocity can be obtained by substituting the value of u given in Eq. (2) into Eq. (1).

$$H_{\mathrm{min}} = d_{\mathrm{p}}(A + \sqrt{BC}) \quad (3)$$

So as long as the same values of A–C can be obtained the minimum value of HETP is directly proportional to particle diameter.

Eqs. (1) and (2) can be used as the basis for determining which approaches may be beneficial in improving operating speed and efficiency in HPLC. From the discussion above it can be seen that benefits could be gained by reducing the size of the stationary phase packings that are employed in pharmaceutical analysis. Smaller particles give the potential for columns with higher optimum linear velocities and at the same time columns whose efficiency is less dependent upon linear velocity.

The main difficulty with using smaller diameter packings is that the pressure required to pump the mobile phase through the column increases with the square of the particle diameter [3]. In order to gain the full benefits of small particles higher operating pressures are required than can be obtained with standard commercial systems.

1.4. Ultra-high-pressure liquid chromatography (UHPLC) and ultra-performance liquid chromatography (UPLC)

1.4.1. UHPLC

Whilst commercial HPLC systems typically have a maximum operating pressure of around 400 bar there has been interest for a number of years in the benefits of higher pressures. Work in academic laboratories has employed non-porous silica and zirconia based packing materials with diameters in the range of 1–1.5 μm, and used pressures which are one order of magnitude greater than those found in HPLC [4–9]. Non-porous particles are used because of their mechanical strength and relative ease of manufacture. In addition, fused silica capillaries with diameters in the range 10–150 μm are used to minimise the impact of frictional heating. In general, higher efficiencies are obtained from narrower capillaries [8,10] with systematic changes to the A and C terms of the van Deemter equation being observed [10]. Because of the very high pressures involved special equipment is required to pump the mobile phase and to pack the columns. The term UHPLC has been used to distinguish this technique from conventional HPLC. The use of UHPLC has given efficiencies of up to 300,000 plates per column for analytes such as hydroquinone [4]. Pharmaceuticals such as benzodiazepines were separated and detected by either UV absorbance or time of flight MS [7]. Gradient UHPLC was also employed to separate the many peptides produced by a tryptic digest of ovalbumin [5].

In pharmaceutical development, the large sample concentration ranges inherent in purity assessment (see above) mean that porous packing materials are normally employed in HPLC. Porous packing materials capable of withstanding higher pressures are now available and they have been shown to give much

higher sample capacity than non-porous packings [11]. There are also reports of these materials being packed into columns with a diameter of 1 mm [12].

1.4.2. UPLC

Because of the need to increase separating efficiency and the clear potential demonstrated by UHPLC work there is now considerable interest from instrument and column manufacturers in elevated pressure HPLC. Commercial systems capable of generating pressures up to 1000 bar are now available and one of the manufacturers (Waters Corporation) uses the term UPLC to describe their approach. The term UPLC is used in this work for the purposes of clarity. Whilst 1000 bar is a modest pressure in comparison to the pressures used in UHPLC it is a significant increase on standard HPLC conditions and so is likely to offer considerable benefit in routine analysis. There are several reports covering the application of UPLC to pharmaceutical analysis [13–16] particularly in the field of metabolism studies [13–15]. UPLC has been shown to generate high peak capacities in short times and these are of particular benefit in analysing the complex mixtures that constitute metabolism samples.

In this work the potential of UPLC to improve the analysis of the samples that are encountered during pharmaceutical development is explored. Particular emphasis has been placed on determining whether UPLC can reduce analysis times without compromising the quantity and quality of the analytical data generated.

2. Experimental

2.1. van Deemter plots

The work on comparing the peak efficiencies of UPLC and HPLC as a function of linear velocity was performed using a beta test versions of the Acquity instrument and a 100 mm × 2.1 mm column packed with 1.7 μm Acquity C_{18} BEH (Milford, USA). All other UPLC work was performed using a commercial instrument and columns (see below). HPLC was performed using an HP 1090 (Stockport, UK) and a 150 mm × 4.6 mm column packed with 5 μm Hypersil advance (Runcorn, UK). The analyte was a 0.025 mg/ml solution of propranolol (Aldrich, UK). The mobile phases were 30% acetonitrile and 70% aqueous 10 mM ammonium formate at pH 4 for UPLC and 25% acetonitrile/50 mM ammonium acetate at pH 4 for HPLC. Efficiency was determined from the retention time and peak width at half height.

2.2. Performance comparisons using AstraZeneca compounds

2.2.1. UPLC

UPLC was performed using a commercial Acquity system from Waters (Elstree, UK) and 100 mm columns with an i.d. of 2.1 mm packed with 1.7 μm Acquity C_{18} BEH particles (Elstree, UK). The UPLC system was equipped with a 500 nl flow cell and a Rheodyne injector. Water was de-ionised using a Millipore system (Waters, Elstree, UK). Acetonitrile and tri fluoro acetic acid (TFA) were from Fisher Scientific (Loughborough, UK). The A solvent was 0.1% TFA in water (v/v) and the B solvent was 0.1% TFA in acetonitrile (v/v). For each experiment, the flow rate was 0.5 ml min^{-1}. Solvent strength was varied linearly as per conditions described in Table 1.

Table 1
UPLC gradient conditions

	Starting condition	Finishing condition	Gradient time (min)
AZ compound 1	15% B	35% B	5
AZ compound 2	10% B	75% B	15
AZ compound 3	10% B	90% B	10

The column was thermo-stated at 35 °C. UV absorbance data were collected at 261 nm with a bandwidth of 1.2 nm for AZ compound 1, 258 nm with a bandwidth of 10.8 nm for AZ compound 2, and 250 nm with a bandwidth of 1.2 nm for AZ compound 3. For all experiments, a data collection rate of 10 Hz was employed. The data were used without digital filtering. For each compound 5 μl injections were made using a 20 μl loop operated in partial loop pressure assisted mode. The sample concentrations were as follows: AZ compound 1 0.3 mg ml^{-1}, AZ compounds 2 and 3 0.1 mg ml^{-1}. The weak needle wash solution was 0.1% (v/v) TFA in water and the strong needle wash solution was 0.1% (v/v) TFA in acetonitrile.

2.2.2. HPLC

HPLC was performed using an Agilent 1100 systems (Agilent, Stockport, UK). Water was de-ionised using a Millipore system (Waters, Elstree, UK). Acetonitrile was from Rathburns (Walkerburn, UK), tri fluoroacetic acid (TFA), propan-2-ol (IPA) were from Fisher Scientific (Loughborough, UK) and heptafluorobutyric acid (HFBA) from Acros (Geel, Belgium). For all experiments, a data collection rate of 2.5 Hz was employed.

2.2.3. AZ compound 1

The column was 150 mm × 4.6 mm i.d. packed with 3 μm Luna phenyl hexyl particles (Phenomenex, Macclesfield, UK). The A solvent was 0.1% TFA in water (v/v), the B solvent was 0.1% TFA in acetonitrile (v/v) and the C solvent was 0.1% TFA in TetraHydroFuran (THF). The flow rate was 1.0 mL min^{-1}. The starting condition conditions were 80% A, 20% B and 0% C and the finishing conditions 40% A, 57% B and 3% C. Solvent strength was varied linearly over 30 min. The column was thermo-stated at 50 °C. UV absorbance data were collected at 261 nm using a bandwidth of 2 nm.

2.2.4. AZ compound 2

The column was 150 mm × 4.6 mm i.d. packed with 3 μm YMC-PACK ODS-AQ particles (YMC, Schermbeck, Germany). The A solvent was 0.01% HFBA, 1% IPA in water (v/v/v), the B solvent was 0.01% HFBA, 1% IPA in acetonitrile (v/v/v). The flow rate was 1.2 ml min^{-1}. The starting conditions were 70% A and 30% B. This was maintained for 7.5 min. Then

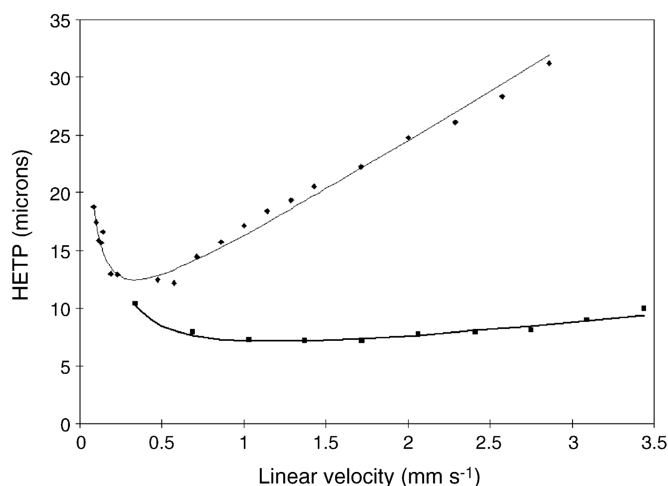

Fig. 1. HETP vs. linear velocity curves for 5 μm particles (diamonds), and 1.7 μm particles (squares).

the solvent strength was varied linearly from the starting conditions (70% A, 30% B) to 64% A and 36% B over 3 min. Then these conditions (64% A and 36% B) were maintained for 6 min. Then the solvent strength was varied linearly from 64% A and 36% B to the final conditions 30% A and 70% B over 8.8 min. The column was thermo-stated at 40 °C. UV absorbance data were collected at 258 nm using a bandwidth of 2 nm.

2.2.5. AZ compound 3

The column was a 150 mm × 4.6 mm i.d. packed with 3.5 μm Zorbax Bonus RP (Agilent, Stockport, UK). The A solvent was 0.1% TFA in water (v/v) and the B solvent was 0.1% TFA in acetonitrile (v/v). The flow rate was 1 ml min^{-1}. The starting conditions were 5% A and 95% B and the finishing conditions 5% A and 95% B. Solvent strength was varied linearly over 60 min. The column was thermo-stated at 35 °C. UV absorbance data were collected at 254 nm using a bandwidth of 2 nm.

2.2.6. Quantification work

UPLC and HPLC experiments were performed using AZ compound 2. The conditions were the same as above.

3. Results and discussion

3.1. Efficiency and linear velocity

From the discussion given in the introduction and analysis of Eqs. (1) and (2) it is expected that the use of smaller particles will lead to van Deemter plots which have lower minima and less curvature at high linear velocities. In addition the curve minima should occur at higher linear velocities. In order to check this expectation the efficiencies of isocratic UPLC and HPLC were determined by analysing the beta blocker. Propranolol using acetonitrile/aqueous buffered mobile phases at pH 4. The HETP values were determined from the peak width at half height, and using mobile phase linear velocities ranging between 0.09 and 3.4 mm s^{-1}. The linear velocity was determined from the elution time of the sample solvent. Typically four separate determinations were made at each linear velocity and the average values used.

The resultant curves for HPLC and UPLC are shown in Fig. 1. From Fig. 1 it can be seen that HETP versus u curves both have the form expected from Eq. (1), but that there are large differences between them. The curve for the 5 μm particles shows a minimum HETP at around 0.32 mm s^{-1} and then rises steeply as the linear velocity is increased. At a linear velocity of 2.8 mm s^{-1} (which corresponds to a flow rate of about 2 ml min^{-1}) the HETP is about two and a half times the minimum value.

The curve for the 1.7 μm particles is much flatter with the HETP at a linear velocity of 3.5 mm s^{-1} (about 0.5 ml min^{-1}) being only about 30% higher than the minimum value. This indicates that, as expected from theory, linear velocity can be increased to reduce analysis times without any significant loss in operating efficiency. Fig. 1 shows that the optimum linear velocity with the 1.7 μm particles is about 1.1 mm s^{-1} as opposed to about 0.32 mm s^{-1} with the 5 μm particles. The ratio of the optimum linear velocities is 3.4 which is in fair agreement to the value of 2.9 expected from the ratio of the particle diameters (see Eq. (2)).

The value of $H_{\min}$ is about 2.5 times the particle size for the 5 μm particles and about 4.1 times the particle size for the 1.7 μm particles. These values imply that the experimental 1.7 μm column was not as well packed as the 5 μm commercial column.

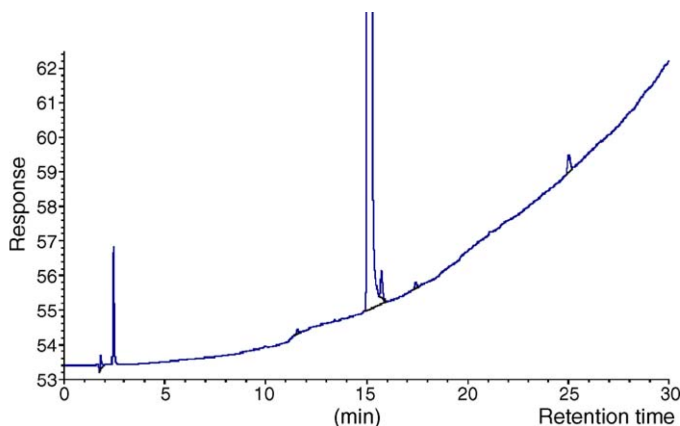

Fig. 2. HPLC analysis of development compound 1.

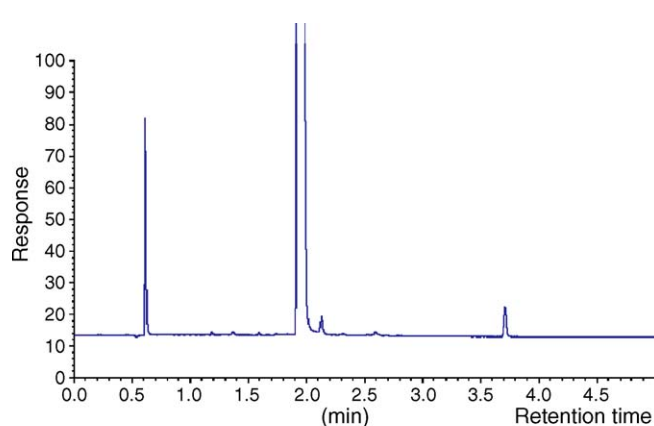

Fig. 3. UPLC analysis of development compound 1.

Subsequent work with commercial 1.7 μm columns has shown significant improvements in efficiency.

3.2. Investigation into the applicability of UPLC in pharmaceutical development

Following the exploratory work with propranolol detailed above, we decided to investigate the potential of UPLC as a tool for the routine analysis of some early pharmaceutical development compounds. UPLC was compared to conventional HPLC in terms of separation quality, separation speed, method development time, data processing times, and data quality. To do these comparisons UPLC methods were developed that gave the same separating power as the existing HPLC method. As a result of earlier work [16] a column length of 100 mm was chosen for all the UPLC separations. The samples were all bases having either aliphatic or aromatic nitrogen atoms, and aromatic functional groups. UPLC showed advantages in each of the development compounds examined. In this work three typical examples have been shown where UPLC has shown significant benefits over conventional HPLC in terms of analysis time, data processing time, and method development time. UPLC also generated data of a similar quality to that typically expected with conventional HPLC.

3.2.1. AZ development compound 1

The HPLC separation of a sample of AZ development compound 1 is shown in Fig. 2. Samples are taken and analysed prior to and after the purification steps in order to determine the efficiency of those steps. The HPLC method employs a reversed phase column packed with 3 μm particles and a gradient elution programme with a flow rate of 1.0 ml min^{-1}. The mobile phase constituents are water, acetonitrile, and THF, each containing 0.1% TFA (v/v). The gradient elution with both acetonitrile and THF is needed to produce the selectivity and peak shape required. Fig. 2 shows that with the conventional HPLC method an analysis time of 30 min is required to separate the impurities of interest.

The UPLC separation of the crude sample of development compound 1 is shown in Fig. 3. The UPLC mobile phase components were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The UPLC conditions are a simple gradient from 15% B to 35% B in 5 min at a flow rate of 0.5 ml min^{-1}. Because of the differences in column diameter the mobile phase linear velocity in UPLC was nearly two and a half times that used in HPLC. From Fig. 3 it can be seen by visual inspection that UPLC gives a separation of at least the same quality as that from HPLC, but in only 5 min rather than thirty. In particular the resolution obtained by UPLC between AZ development compound 1 and the low level component eluting immediately after it, is at least as good as that seen by HPLC. The UPLC separation also showed benefits in terms of a flatter baseline and simpler mobile phase and gradient programme. Examination of the chromatogram of a solvent blank (not shown) showed that there were no interfering peaks from the reagents or the UPLC system.

3.2.2. AZ development compound 2

UPLC was used in conjunction with HPLC to assess the quality of material produced during the development of a new synthetic route to produce AZ development compound 2. As part of this work the photolytic stability of material produced by the different experimental routes was assessed. The samples were screened by the existing HPLC method and a UPLC method. Fig. 4 shows the results from the analysis of one such sample of light degraded material by the existing HPLC method. The HPLC method uses a 150 mm column packed with 3 μm particles and a mobile phase containing water, acetonitrile, hepta fluoro butyric acid (HFBA), and iso-propyl alcohol with a flow rate of 1.2 ml min^{-1}. The HFBA was used in place of the more normal TFA in order to achieve the desired selectivity. A complex four steps gradient and an analysis time of 30 min was necessary to produce the separation required. The light-degraded sample contains a large number of components with many of them eluting towards the end of the gradient. These later eluting components are shown in more detail in the expansion inset into Fig. 4.

The results of the UPLC analysis of the same sample of light-degraded development compound 2 are shown in Fig. 5. Again, the inset in Fig. 5 shows an expansion of the later part of the gradient. The UPLC method has a mobile phase composed of acetonitrile, water, and TFA and a simple gradient from 10 to 75% organic in 15 min. A visual comparison of Figs. 4 and 5 shows that the UPLC method gives sharper peaks. The main

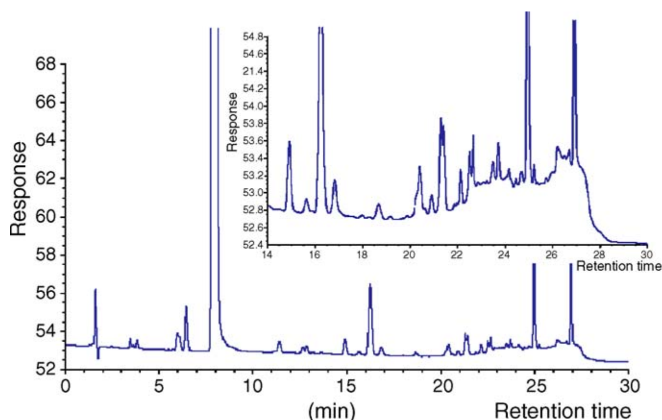

Fig. 4. HPLC analysis of development compound 2.

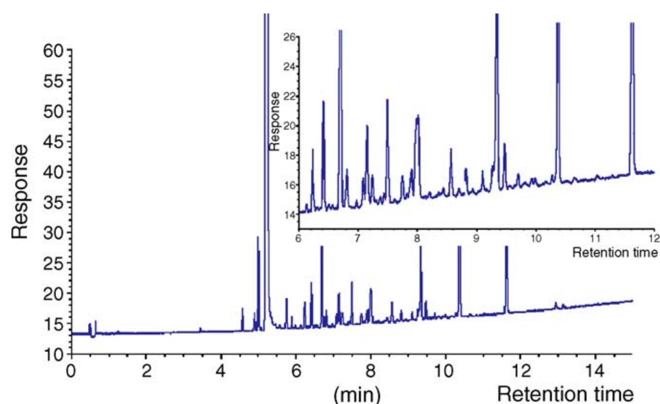

Fig. 5. UPLC analysis of development compound 2.

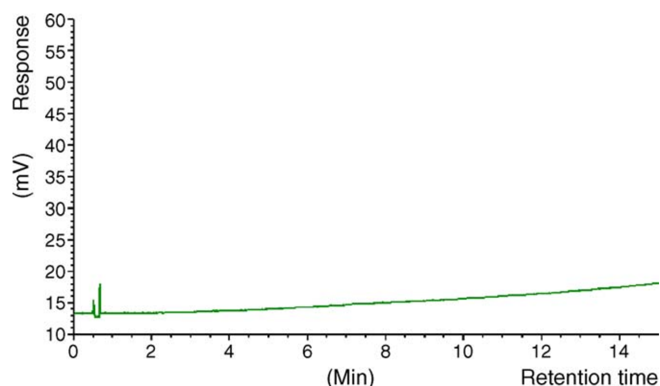

Fig. 6. UPLC blank injection.

degradation products and impurities separated by the UPLC method were identified by UPLC/MS and compared with data known from existing methods including HPLC/MS. The results showed that all of the major degradation products and impurities were separated by the UPLC approach but in half of the time required by the HPLC. The UPLC method was also beneficial as it gave a flatter baseline, sharper peaks, and very good retention time precision. This meant that less time was required to integrate the UPLC data than the HPLC data as less manual intervention was needed. Additional benefits were a simpler gradient programme and mobile phase composition. Examination of the chromatogram of a solvent blank, shown in Fig. 6, demonstrated that there were no interfering peaks from the reagents or the UPLC system. The ability to replace HFBA with the more commonly used TFA also made it easier to interpret the MS data. An additional benefit with the UPLC method is that the mobile phase consumption was only about 20% of that of the HPLC method.

3.2.3. AZ development compound 3

Development compound 3 is at a very early stage of development and an early sample was assessed by both conventional HPLC and UPLC. Fig. 7 shows the results of the analysis of a crude sample of development compound 3 using a generic HPLC gradient method. The HPLC method uses 0.1% TFA in water and 0.1% TFA in acetonitrile as mobile phase components a linear gradient from 5 to 95% organic in 60 min with a flow rate of 1.0 ml min^{-1}. The column has a length of 150 mm and uses 3.5 μm particles. Fig. 7 indicates the presence of an impurity eluting immediately before the active substance and a number of other impurities eluting through the gradient.

The UPLC analysis on the same sample of AZ development compound 3 is shown in Fig. 8. The UPLC method was developed using the same mobile phase system as the HPLC method and a flow rate of 0.5 ml min^{-1}. The UPLC flow rate gives a linear velocity that is nearly two and a half times that employed in the HPLC method. Comparison of Figs. 7 and 8 shows that the UPLC method separates the same number of impurities as the HPLC method but in only 10 min rather than 60. This reduction in analysis time has been achieved without loss of resolution between the impurities. For example, both UPLC and HPLC give baseline resolution between AZ development compound 3 and the impurity eluting just before it. Again, the UPLC method benefits from a much flatter baseline and so easier integration of the impurity peaks. Method development for UPLC simply entailed varying the starting and finishing percentage of organic modifier, and the gradient time. The high linear velocities and rapid column re-equilibration meant that the UPLC method was developed in under 2 h. The chromatogram from the solvent blank did not show any interfering peaks.

3.2.4. Performance of UPLC in routine operation

The performance of UPLC in routine operation was determined by measuring injector precision, performance in an external standard analytical run, and comparing results to those obtained in HPLC. In each of the tests, samples of development compound 2 were analysed using the UPLC method given above.

For the precision tests a degraded sample of development compound 2 was injected 10 times and the retention time and peak area precisions were determined for six of the impurities. The concentrations of the impurities varied from 0.03 to 2.87%. The retention time precision data are shown in Table 2 and the peak area precision data in Table 3. From Table 2, it is seen that the retention time precision is very good for all of the impurities. In Table 3, it is seen that the peak area precision varies from less than 1% RSD with an impurity at the 2.87% level to nearly 10% RSD with an impurity at the 0.03% level. These precision data are satisfactory for the purpose of the analytical method.

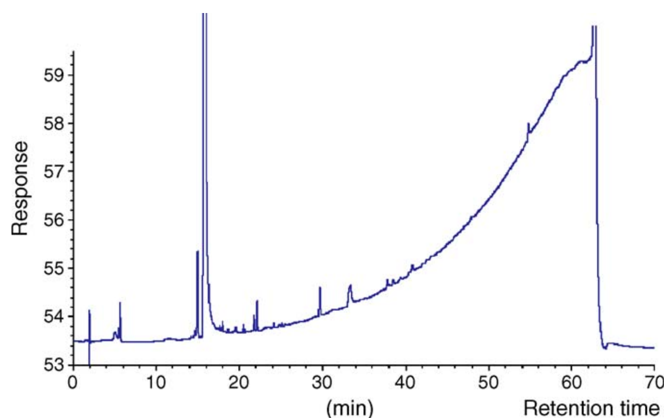

Fig. 7. HPLC analysis of development compound 3.

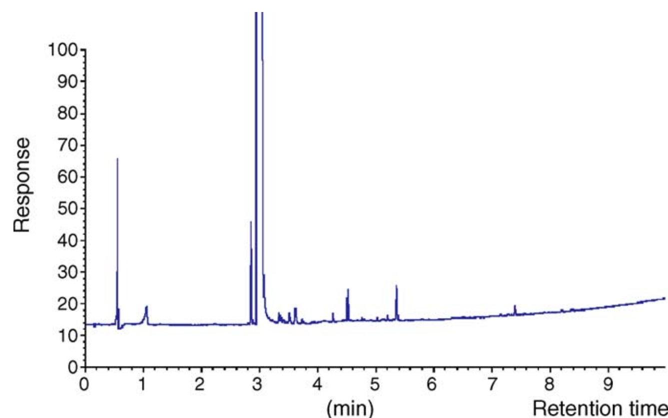

Fig. 8. UPLC analysis of development compound 3.

Table 2
Retention time precision

Replicate	Peak name					
	A	B	C	D	E	F
1	4.95	5.46	7.75	6.70	9.31	3.86
2	4.95	5.46	7.75	6.70	9.31	3.87
3	4.95	5.46	7.75	6.70	9.32	3.87
4	4.95	5.46	7.75	6.70	9.32	3.87
5	4.95	5.46	7.75	6.70	9.32	3.87
6	4.95	5.46	7.75	6.70	9.31	3.87
7	4.95	5.46	7.75	6.70	9.31	3.87
8	4.95	5.46	7.75	6.70	9.32	3.87
9	4.95	5.46	7.76	6.70	9.32	3.87
10	4.95	5.46	7.76	6.70	9.32	3.87
Average	4.95	5.46	7.75	6.70	9.32	3.87
% RSD	0.00	0.00	0.05	0.00	0.06	0.08

In the external standard test a degraded sample of development compound 2 was run against a sample of the analytical standard and a diluted standard equivalent to 0.5% of the nominal sample concentration. A linear detector response with concentration was found in the range 0.5–150 $\mu g\,ml^{-1}$,with a limit of quantification of 0.05 $\mu g\,ml^{-1}$ and a limit of detection of 0.02 $\mu g\,ml^{-1}$. The limit of detection was determined by a comparison of the response of a dilute sample with the average baseline noise. The limit of detection is three times the baseline noise, and the limit of quantification was defined as two and a have times the limit of detection. The USP precision result was typically less than 1% RSD and the standard recovery result typically within 1%. Standard recovery was determined by comparing the responses of two independently prepared standards with their respective weights.

In the final test both UPLC and HPLC methods were employed to analyse the same degraded sample of development compound 2. The levels of the active agent and the impurities were determined by the two techniques and some of the comparative data are shown in Table 4. In the HPLC method, the three impurities listed had relative retention times of 0.86, 1.11, and 0.74. In the UPLC method the relative retention times for the three impurities were 0.91, 1.08, and 0.79, respectively. From Table 4, it is seen that very similar levels of the active and the impurities were found by both UPLC and HPLC methods. With HPLC impurity 3 was detected but at a level below the limit of reliable quantification.

Table 3
Peak area precision

Replicate	Peak name					
	A	B	C	D	E	F
1	125.177	48.301	20.352	12.229	7.164	1.192
2	123.067	47.637	20.579	11.152	7.454	1.060
3	121.838	47.345	20.323	10.970	7.810	1.237
4	124.284	47.624	20.631	11.027	7.705	1.145
5	124.433	48.504	20.733	11.219	7.815	1.180
6	124.072	48.937	20.640	11.037	7.273	1.227
7	123.053	47.892	19.820	10.701	7.520	1.083
8	122.094	47.094	20.036	10.829	7.439	1.466
9	124.873	48.838	21.150	10.940	8.049	1.084
10	123.621	47.277	20.846	11.064	7.949	1.241
Average	123.65	47.94	20.51	11.12	7.62	1.19
% RSD	0.91	1.38	1.90	3.76	3.85	9.85
% Area	2.87	1.11	0.47	0.26	0.18	0.03

Table 4
A comparison of quantitative results by HPLC and UPLC

Component	Component level (% w/w)		
	HPLC	UPLC operator 1	UPLC operator 2
Active	97.5	96.9	97.9
Impurity 1	0.65	0.65	0.65
Impurity 2	0.38	0.34	0.35
Impurity 3	<0.05	0.06	0.06

4. Conclusions

The use of a commercially available elevated pressure HPLC system has been shown to be beneficial in the pharmaceutical development area. In our hands we have been able to obtain faster analysis than that achieved with conventional HPLC, and without sacrificing separating power. Elevated pressure HPLC was shown to be capable of giving good retention time and peak area precision, and has given comparable data to that from conventional HPLC.

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References

[1] U.D. Neue, HPLC Columns, Wiley-VCH, New York, 1997.
[2] J.C. Giddings, Unified Separation Science, Wiley, New York, 1991.
[3] L. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, Wiley, New York, 1979.
[4] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 983–989.
[5] J.E. MacNair, K.D. Patel, J.W. Jorgenson, Anal. Chem. 71 (1999) 700–708.
[6] K. Lan, J.W. Jorgenson, Anal. Chem. 71 (1999) 709–714.
[7] J.A. Lippert, B. Xin, N. Wu, M.L. Lee, J. Microcolumn Sep. 11 (1997) 631–643.
[8] N. Wu, J.A. Lippert, M.L. Lee, J. Chromatogr. A 911 (2001) 1–12.
[9] Y. Xiang, B. Yan, C.V. McNeff, P.W. Carr, M.L. Lee, J. Chromatogr. A 1002 (2003) 71–78.
[10] K.D. Patel, A.D. Jerkovich, J.C. Link, J.W. Jorgenson, Anal. Chem. 76 (2004) 5768–5777.
[11] J.S. Mellors, J.W. Jorgenson, Anal. Chem. 76 (2004) 5441–5450.
[12] L.A. Colón, J.M. Cintrón, J.A. Anspach, A.M. Fermier, K.A. Swinney, Analyst 129 (2004) 503–504.
[13] R. Plumb, J. Castro-Perez, J. Granger, I. Beattie, K. Joncour, A. Wright, Rapid Commun. Mass Spectrom. 18 (2004) 2331–2337.
[14] J. Castro-Perez, R. Plumb, J. Granger, I. Beattie, K. Joncour, A. Wright, Rapid Commun. Mass Spectrom. 19 (2005) 843–848.
[15] I.D. Wilson, R. Plumb, J. Granger, H. Major, R. Williams, E.M. Lenz, J. Chromatogr. B 817 (2005) 67–76.
[16] S.A.C. Wren, J. Pharm. Biomed. Anal. 38 (2005) 337–343.