

The Evaluation and Application of UPLC for the Rapid Analysis of Dose Formulations



The use of Ultra Performance Liquid Chromatography (UPLC™) with UV and MS detection was evaluated in several pharmaceutical applications. Initial studies characterized the commercial instrumentation performance parameters for isocratic and gradient separations as a function of flow rate using several commercially available drugs as model probe compounds. Parameters examined included reproducibility of retention time and peak area, as well as theoretical plates, resolution, and column ruggedness. The use of high-speed separations for dose formulation strength analysis was evaluated using two model drug compounds: mefenamic acid and chloramphenicol, in a dimethylacetamide/polyethyleneglycol-200 vehicle. Accuracy, precision, and resolution were examined under high-speed gradient conditions for the dose formulation analysis.

The pharmaceutical industry is under intense pressure to increase productivity and put new drugs onto the market in a shorter time period (1). Various approaches including high-throughput screening, combinatorial chemistry, proteomic/genomic target identification techniques, high-throughput in vitro screening to determine physicochemical and absorption/distribution/metabolism/elimination properties of compounds, rapid in vivo pharmacokinetic screening, and the use of biomarkers and pharmacogenomics are being employed in drug discovery and development activities. A common theme in these approaches is the need to provide high-quality data at a faster rate to drive decision making processes.

Analytical chemists are challenged to find faster ways of delivering quality data across a range of project-driven needs. A number of approaches are being employed to increase separation throughput including dual high performance liquid chromatography (HPLC) column switching techniques (2,3), direct injection approaches for biological samples (4,5), high-speed supercritical fluid chromatography (6), parallel (96-capillary) capillary electrophoresis (7,8), parallel (24-lane) HPLC (9,10), monolithic HPLC columns (11,12), and ultrahigh performance chromatography (UPLC) (13–17). UPLC was pioneered in the late 1990s by the Jorgenson (13–15) and Lee (16,17) groups and typically involves the use of 1–2 mm particles at much higher pressures

(15,000–100,000 psi) than conventional HPLC instruments (4000–6000 psi). The use of columns packed with 1-mm particles in combination with high pressures allows dramatic decreases in analysis time with little compromise in column performance. The relatively flat nature of the van Deemter plot for these small particles at higher linear velocities accounts for the ability to operate at high flow rates without severe effects on column efficiency (13,15). Recently, commercial instrumentation capable of operating up to 15,000 psi combined with columns packed with 1.7 mm particles has become available in the form of the Waters® ACQUITY UPLC™ system. This report describes the basic performance of this commercial instrumentation, as well as pharmaceutical applications for the rapid analysis of dose formulations.

Experimental

ACQUITY UPLC Performance

Characterization: *Isocratic Conditions:* An ACQUITY UPLC system (Waters, Milford, Massachusetts) equipped with a UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) was evaluated under isocratic conditions using a water/acetonitrile/formic acid (65/35/0.1; v/v/v) mobile phase pumped at flow rates from 0.1 to 1.2 mL/min. The column temperature, data sampling rate, filter constant, injection volume, and detection wavelength were 55 °C, 20 pts/s, fast, 5 mL and 233 nm, respectively. A mixture of NAP (10 mg/mL) and KR (100 mg/mL) in

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Table I: Gradient conditions

Gradient	Time (min)	Flow (mL/min)	%A	%B
Standard	0.00	0.50	75	25
	2.00	0.50	0	100
	2.10	0.50	75	25
Fast	0.00	1.00	75	25
	0.50	1.00	0	100
	0.51	1.00	75	25

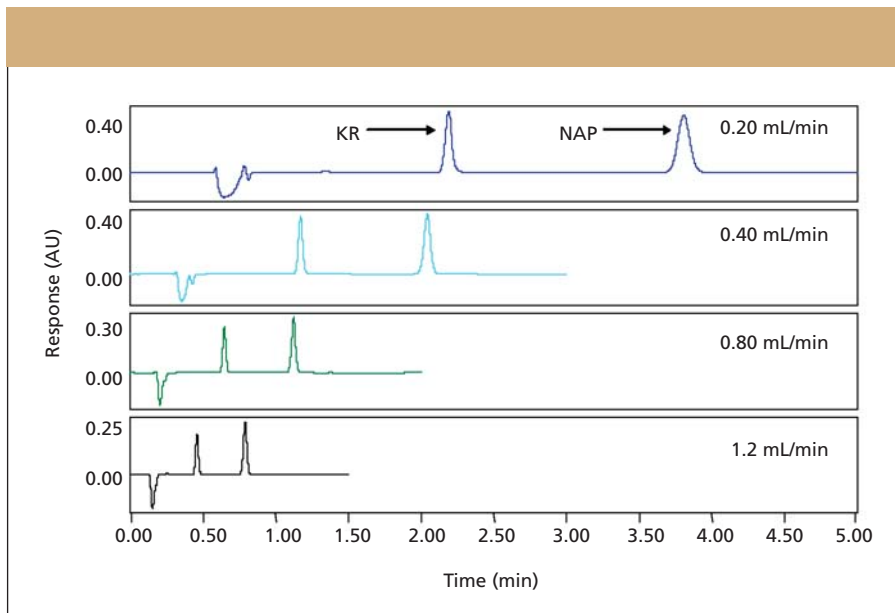


Figure 1: Representative chromatograms obtained under isocratic conditions at various flow rates for a combined Ketorolac/Naproxen standard using UPLC.

Table II*: Ketorolac — Average (%RSD), Retention Time, Peak Area, Peak Height, and Theoretical Plates (N) as a Function of Flow Rate (n = 3 injections at each flow rate)

Flow Rate (mL/min)	t_r , min Avg (%RSD)	Peak Area $\times 10^4$ Avg (%RSD)	Peak Ht $\times 10^3$ Avg (%RSD)	N Avg (%RSD)
0.10	4.159 (0.06)	315 (0.28)	445 (0.38)	8878 (1.00)
0.20	2.188 (0.03)	157 (0.23)	485 (0.71)	13257 (1.12)
0.30	1.513 (0.04)	105 (0.04)	450 (0.23)	14136 (0.44)
0.40	1.171 (0.05)	79.3 (0.03)	399 (0.39)	14196 (0.62)
0.50	0.965 (0.00)	62.9 (0.22)	366 (0.26)	13261 (0.41)
0.60	0.824 (0.07)	52.1 (0.17)	326 (0.28)	12186 (0.78)
0.70	0.724 (0.00)	44.4 (0.13)	296 (0.13)	11465 (0.84)
0.80	0.646 (0.09)	38.8 (0.23)	270 (0.34)	10801 (0.96)
0.90	0.585 (0.20)	34.4 (0.24)	247 (0.20)	10091 (0.41)
1.0	0.537 (0.11)	30.4 (0.65)	225 (0.50)	9447 (0.45)
1.1	0.496 (0.00)	27.5 (0.47)	209 (0.47)	8994 (0.47)
1.2	0.461 (0.13)	25.1 (0.54)	195 (0.57)	8374 (0.67)

* t_r = Retention Time, Peak Ht = peak height, and N = Theoretical Plates

MeOH was injected in triplicate at each flow rate. The average and %RSD was calculated for the retention time, peak area, peak height, theoretical plates, and resolution under each flow condition. Additionally, over 4000 injections were performed under a high pressure conditions (1.25 mL/min; 13,500 psi) to test the column ruggedness. Interspersed among the 4,000

injections were runs done at 0.5 mL/min that were used to assess the column performance.

Gradient Conditions: An ACQUITY UPLC system equipped with an UPLC BEH C18 column (2.1×50 mm, $1.7 \mu\text{m}$) was evaluated under a standard and a fast gradient condition (see Table 1). For both gradient conditions, mobile phase A was

water with 0.1% formic acid (v/v), mobile phase B was acetonitrile with 0.1% formic acid (v/v) and the column temperature, data sampling rate, filter constant, detection wavelength and injection volume were 50°C , 20 pts/s, fast, 277 nm and 5 mL, respectively. Replicate injections (n = 5) of a test sample composed of 50 mg/mL each of MFA and CA and 75 mg/mL NAP in water/MeOH (50/50; v/v) was used to examine performance under each gradient condition.

Rapid dose formulation analysis: A model dose formulation containing both MFA (1.410 mg/mL) and CA (0.856 mg/mL) was prepared in DMAC/PEG200 (10/90; v/v). Replicate (n = 3) aliquots (1 mL) of the dose formulation was transferred to separate 25-mL volumetric flasks and each was diluted to volume with water/MeOH (50/50; v/v). A series of standards containing both MFA and CA at the 10, 25, 50, and 100 mg/mL levels were prepared by serial dilution of a combined 1 mg/mL stock of each compound using water/MeOH (50/50; v/v) as the diluent. The diluted dose formulations and each standard were injected in replicate (n = 5) on the system using the two gradient conditions described in Table I. The concentration of MFA and CA in the dose formulation was determined by interpolation from a standard curve constructed using the first injection of each standard for the curve construction.

Results and Discussion

UPLC System Performance: *Isocratic analysis:*

A standard solution containing KR and NAP as model drug compounds was examined by UPLC under isocratic conditions to determine the instrument performance as a function of flow rates from 0.1 to 1.2 mL/min. Representative chromatograms for low, mid, and high flow rate conditions are shown in Figure 1. Symmetrical peak shapes were obtained under all flow conditions and KR and NAP were well resolved under all conditions. The average values and reproducibility (%RSD) for the retention time, peak area, peak height, theoretical plates (N), and resolution obtained for three replicate injections under each flow condition are shown in Tables II and III for KR and NAP, respectively. The RSD values obtained for retention time reproducibility were, in general, less than 0.1%. The peak area and peak height reproducibility were, in general, less than 1% RSD and most values ranged between 0.1 and 0.4% RSD. Theo-

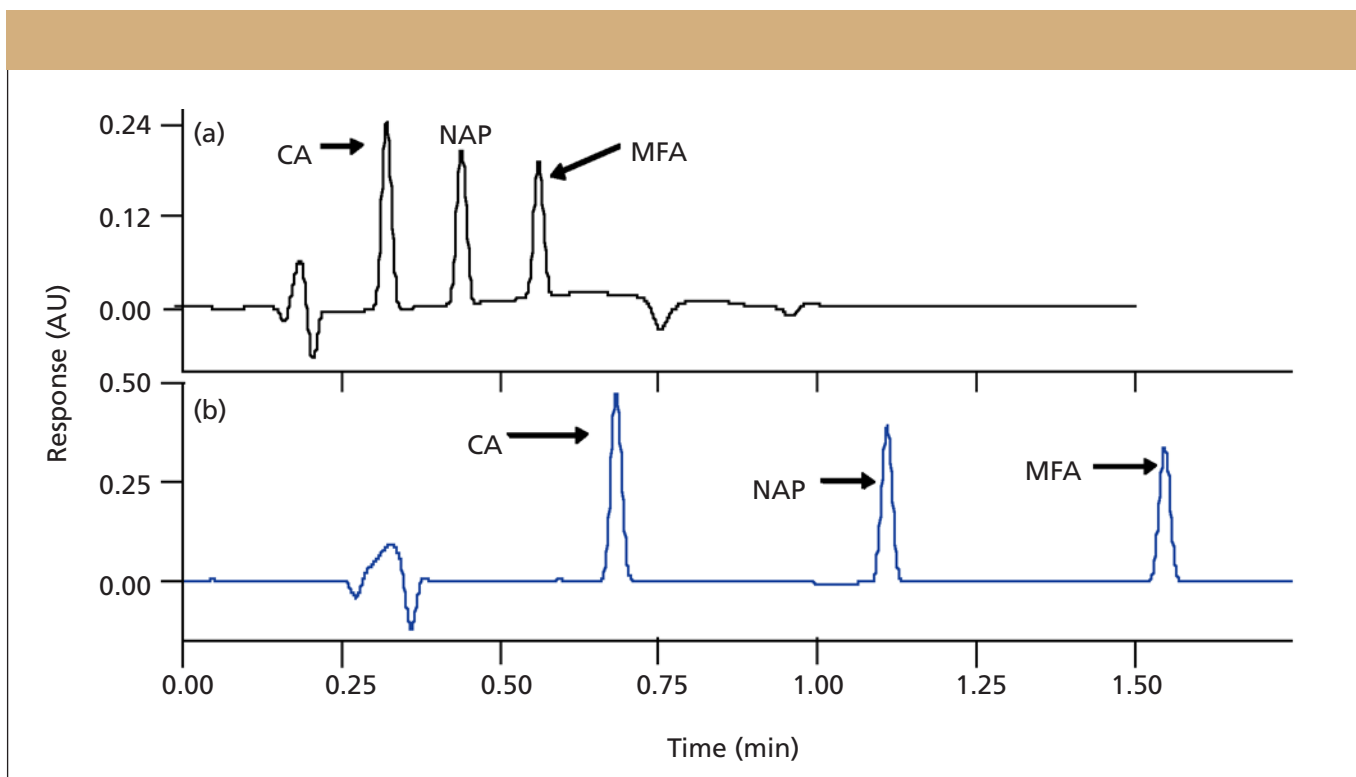


Figure 2: Representative chromatograms for a combined chloramphenicol/mefenamic acid obtained under: (a) fast and (b) standard gradient UPLC conditions.

retical plates decreased by 41 and 26% for KR and NAP, respectively, when operating at the highest flow rate, 1.2 mL/min, relative to the optimal values found at 0.3–0.4 mL/min. Similarly, there was a 17% decrease in resolution when operating at the highest flow rate relative to the optimal flow rate. This small decrease in resolution is contrasted with the four-fold decrease in analysis time.

The long-term performance of the UPLC BEH C18 column was determined by performing 4000 isocratic injections of the

KR/NAP standard at high pressure (~13,500 psi, 1.25 mL/min). The column performance was periodically monitored during the injections by analyzing the KR/NAP standard at a lower flow rate (0.5 mL/min). The retention times, peak shapes, theoretical plates, and the resolution were found to remain constant throughout the 4000 injections (data not shown).

Gradient analysis: The performance of the UPLC instrument under standard (0.5 mL/min flow rate with 2 min linear ramp from initial to final mobile phase con-

dition) and fast (1.0 mL/min flow rate with 0.5 min linear ramp from initial to final mobile phase condition) gradient conditions were examined using a probe sample containing CA (50 mg/mL), NAP (75 mg/mL), and MFA (50 mg/mL). Representative chromatograms for the standard and fast gradient conditions are shown in Figure 2. All three peaks are well resolved under both gradient conditions with the last peak, MFA, eluting at 1.54 and 0.56 min for the standard and fast gradient, respectively. Using the fast gradient conditions, an injection-to-injection time of 1 min could be achieved. The average value and %RSD obtained for the retention time, peak area, and resolution for each compound are shown in Table IV. The RSD values for the retention time of all three compounds for the standard and fast gradient methods were less than 0.07% and 0.30%, respectively. The peak area RSD values for all compounds under standard and fast gradient conditions were less than 0.70%. Resolution decreased from a value of 13.3 under the standard conditions to 4.10 under the fast gradient conditions but as seen in Fig 2 all peaks were well resolved under the fast gradient conditions.

Table III*: Naproxen — Average (%RSD), Retention Time, Peak Area, Peak Height, Theoretical Plates (N), and Resolution as function of Flow Rate (n = 3 injections at each flow rate)

Flow Rate (mL/min)	t_r , min Avg (%RSD)	Peak Area $\times 10^4$ Avg (%RSD)	Peak Ht $\times 10^3$ Avg (%RSD)	N Avg (%RSD)	Resolution Avg (%RSD)
0.10	7.202 (0.08)	533 (0.30)	386 (0.99)	6320 (0.10)	11.2 (0.10)
0.20	3.806 (0.03)	267 (0.21)	449 (0.21)	10287 (0.95)	14.3 (0.37)
0.30	2.639 (0.04)	178 (0.13)	434 (0.11)	11650 (0.51)	15.2 (0.19)
0.40	2.043 (0.05)	133 (0.17)	413 (0.38)	12185 (0.75)	15.6 (0.31)
0.50	1.686 (0.03)	1064 (0.11)	400 (0.20)	11869 (1.13)	15.3 (0.44)
0.60	1.440 (0.07)	87.9 (0.12)	366 (0.23)	11438 (0.78)	15.0 (0.33)
0.70	1.262 (0.05)	75.3 (0.11)	346 (0.13)	11047 (0.38)	14.7 (0.35)
0.80	1.124 (0.00)	65.9 (0.15)	325 (0.27)	10689 (0.42)	14.4 (0.34)
0.90	1.015 (0.10)	58.1 (0.22)	305 (0.24)	10173 (0.49)	14.0 (0.16)
1.0	0.928 (0.00)	51.1 (0.83)	283 (0.73)	9762 (0.27)	13.7 (0.08)
1.1	0.853 (0.07)	46.2 (0.76)	267 (0.75)	9361 (0.46)	13.3 (0.10)
1.2	0.791 (0.07)	42.3 (1.00)	254 (1.11)	8981 (0.47)	12.9 (0.13)

* t_r = Retention Time, Peak Ht = Peak Height and N = Theoretical Plates.

Rapid Dose Formulation Analysis

The analysis of CA and MFA in the

Table IV*: Performance of UPLC versus gradient conditions (n = 5 injections)

STANDARD CONDITION								
Chloramphenicol (50 mg/mL)			Naproxen (75 mg/mL)			Mefenamic Acid (50 mg/mL)		
t _r , min	Peak Area	t _r , min	Peak Area	Res	t _r , min	Peak Area	Res	
Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)
0.682 (0.066)	535530 (0.37)	1.107 (0.076)	499236 (0.44)	12.85 (0.061)	1.545 (0.029)	616132 (0.58)	13.30 (0.022)	
*Res = Resolution for adjacent pairs: Chloramphenicol/Naproxen and Naproxen/Mefenamic Acid.								
FAST CONDITION								
Chloramphenicol (50 mg/mL)			Naproxen (75 mg/mL)			Mefenamic Acid (50 mg/mL)		
t _r , min	Peak Area	t _r , min	Peak Area	Res	t _r , min	Peak Area	Res	
Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)	Avg (%RSD)
0.323 (0.28)	252980 (0.63)	0.441 (0.28)	232183 (0.51)	4.00 (0.17)	0.562 (0.26)	284657 (0.66)	4.10 (0.25)	
*Res = Resolution for adjacent pairs: Chloramphenicol/Naproxen and Naproxen/Mefenamic Acid.								

DMAC/PEG200 dose formulation was done under the standard and fast gradient conditions for three replicate dilutions of the dose formulation using replicate (n = 5) injections for each dilution. The concentrations of CA and MFA in the diluted dose formulations were determined by interpolation from a linear regression standard curve (Table V). Very similar results were obtained by the standard and fast gradient approach in terms of the found concentration, although the data for the fast analysis was slightly more variable than the standard conditions but was still very acceptable for drug discovery efforts.

Conclusions

The ACQUITY UPLC System was shown to provide accurate and reproducible results for rapid isocratic and gradient analysis of drug molecules in dose formulations. In the

rapid isocratic mode, retention times, peak shapes, theoretical plates, and resolution for a multicomponent drug standard were found to remain constant over 4000 injections. Using fast gradient conditions, injection-to-injection cycle times of 1 min could be obtained, while still maintaining acceptable within-specification results for repeatability for both peak retention time and area. The utility of the UPLC system for the rapid, accurate and precise analysis of drugs in dosage formulations was demonstrated using a model drug system. The UPLC system provided the same accuracy, precision, and ruggedness as a standard HPLC system but allowed decreased analysis times resulting in higher sample throughput.

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Table V: Dose formulation analysis

Dilution	CA		MFA	
	Avg [Found], mg/mL (%RSD)		Avg [Found], mg/mL (%RSD)	
	Standard	Fast	Standard	Fast
1	1.403 (0.12)	1.400 (0.35)	0.850 (0.96)	0.845 (1.22)
2	1.404 (0.10)	1.414 (0.26)	0.858 (0.38)	0.862 (0.38)
3	1.399 (0.16)	1.422 (0.14)	0.845 (0.63)	0.853 (1.03)
Average (%RSD)	1.402 (0.16)	1.412 (0.79)	0.851 (0.75)	0.853 (1.03)