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High-throughput determination of OROS® drug release rate profile using micro parallel liquid chromatography

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Abstract

A high throughput method with the use of micro parallel liquid chromatography (μPLC) technique was first applied for the determination of drug release profiles in OROS® tablets. Currently, high-performance liquid chromatography (HPLC) is a preferred analytical tool to analyze samples released from OROS® tablets. However, it usually takes more than 20 h to analyze a large number of release rate samples and generate a release-rate profile. In this study, with the use of a 24-column Brio cartridge, the μPLC enabled simultaneous analysis of 24 release-rate samples. The total analysis time including the generation of the release-rate profile was greatly reduced to 3 h. Two different OROS® formulations were used to compare the drug release testing using both μPLC and conventional HPLC. The drug release profiles generated using μPLC were comparable with those obtained by HPLC. In addition, the reproducibility and sensitivity of μPLC analysis were examined. Overall, significant reductions in analysis time and solvent consumption were the major advantages of using μPLC in profiling the drug release rate for a controlled-release dosage.

Keywords: OROS® technology; Controlled release; Drug release testing; Micro parallel liquid chromatography (μPLC); HPLC; Comparison

1. Introduction

OROS® technology is designed for patterned and programmed drug delivery. It employs osmosis to provide precise, controlled drug delivery for up to 24 h [1–3]. OROS® technology can be used in a wide range of compounds, including poorly soluble and highly soluble drugs [4,5]. Several drug products incorporating OROS® technology have been available on the market. For example, Concerta® (methylphenidate HCl) is a once-daily extended-release tablet for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in patients aged 6 and older, while Ditropan XL® (Oxybutynin chloride) extended-release tablet is for the once-a-day treatment of overactive bladder characterized by symptoms of urge urinary incontinence, urgency, and frequency.

Unlike a conventional tablet, the OROS® tablets are nondisintegrating osmotically driven tablets that release drug over a period of time, the controlled release dosage forms must have the ability to maintain therapeutic levels of drug with narrow fluctuations. For different OROS® tablets and special dosage forms, drug release testing is essential for the biopharmaceutical characterization of the drug product, and as a tool to assure consistent product quality with a defined set of specification criteria [6].

A United States Pharmacopeia (USP) Type VII apparatus is used to monitor the drug released per specified time interval. In order to prevent the interference from excipients in OROS® tablets, high performance liquid chromatography (HPLC) is used to analyze release rate samples. However, it usually takes a long time to complete HPLC analysis on a large number of samples. For example, to analyze a set of 144 release rate samples (12 tablets released at 12 intervals), HPLC analysis requires up to 20 h. In order to speed up sample analysis, parallel HPLC became one of the most promising approaches, particularly when analyzing a large number of samples under identical chromatographic conditions. In this study, high-throughput micro parallel liquid chromatography (μPLC) was first applied to drug release assay.

The μPLC from Nanostream, Inc. incorporates a 24-column cartridge, which enables 24 parallel liquid chromatographic separations. It offers a novel approach to achieve high-throughput analysis. Since its first introduction in 2004, the μPLC system

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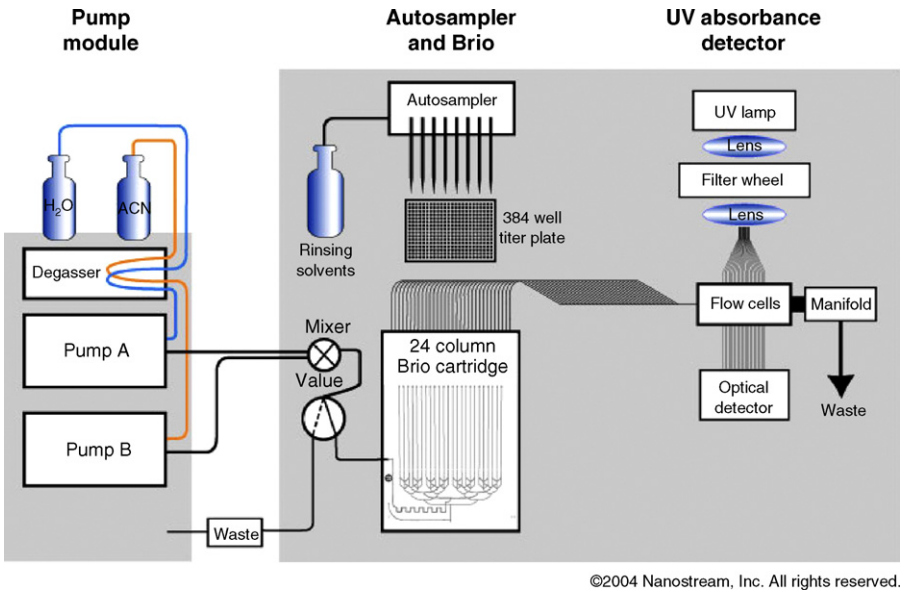

Fig. 1. μPLC system block diagram (with permission from Nanostream, Inc.).

has become a very useful instrument for several applications in drug discovery and development, and particularly for those applications that benefit from higher throughput [7–9]. Recently, μPLC systems have been used for the assessment of compound purity, stability, solubility and other physicochemical properties (e.g., log of the partition coefficient [log *P*], chromatographic hydrophobicity index [CHI]) for a large number of compounds. The aim of our study was to evaluate the feasibility of a μPLC system to analyze the release rate samples in comparison with the analyses by a conventional HPLC method. Using the μPLC as well as conventional HPLC, we performed drug release testing on two different OROS® formulations, OROS® Push-Pull™ (Drug A) and OROS® Push-Stick™ (Drug B). The drug release profiles generated from μPLC were compared to those obtained using conventional HPLC. The μPLC system was also evaluated by comparison to a conventional HPLC system for total analysis time, total solvent consumption, sensitivity, and reproducibility.

2. Materials and methods

2.1. Materials

Ammonium formate and HPLC-grade acetonitrile and methanol were all purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ), and 85% phosphoric acid was obtained from EMD Chemicals, Inc. (Gibbstown, NJ). HPLC-grade water was obtained from a Millipore Milli-Q Gradient purification system (Bedford, MA). A BDS Hypersil C18 column (50 mm × 4.6 mm i.d., 5 μm) was purchased from Thermo Electron Corporation (Bellefonte, PA).

2.2. Equipment

A Vankel Bio-Dis drug release apparatus was purchased from Varian, Inc. (Cary, NC). The Bio-Dis system consists of four components: a water bath, VK-750D heater/circulator, peristaltic pump, and VK-8000 auto sampler. HPLC analyses were run on an Agilent 1100 HPLC system (Agilent Technologies, Inc. Palo Alto, CA), which was equipped with a quaternary pump, a column heater, an auto-injector, and a variable wavelength detector.

A Nanostream LC™ μPLC system and Brio 4208 cartridge (packed with Genesis C18, 80 mm × 0.5 mm, 7 μm) were all obtained from Nanostream, Inc. (Pasadena, CA). A schematic of the system is shown in Fig. 1. The solvent delivery system consists of two solvent pumps that can deliver either isocratic or binary gradient mobile phase to 24-column cartridges. Flow from the solvent delivery system is split evenly across all 24 channels, and results in 1/24th of the programmed pump flow rate flowing through each separation column (i.e., a programmed flow rate of 300 μL/min leads to 12.5 μL/min flowing through each column).

The Brio cartridge has 24 parallel microfluidic columns, each with its own individual sample introduction and exit port. The columns have a rectangular cross section of 1 mm width and 0.2 mm height. These dimensions correspond to a circular cross section of 0.5 mm for comparison with traditional LC columns. The columns are packed with a C18 stationary phase. Samples are introduced onto the Brio cartridge by a 6-channel autosampler from standard 96-wellplates. The injection volume is controlled by a syringe pump, with which samples are quantitatively aspirated from the microwell plate and injected into the Brio well. After separation, samples elute from each column through an exit port that leads to an individual ultraviolet (UV) detector.

2.3. Methods

2.3.1. Drug release measurement

A USP Type VII drug release apparatus (Vankel Bio-Dis) was used to monitor the drug released per specified time interval. Test tubes containing release media (50 mL) were placed into the Bio-

Dis temperature controlled water bath at 37 °C. OROS® drug systems were placed in the sample holders secured on an agitator shaft that was moved by the carriage along the water bath. When programmed, the carriage assembly of the bath dipped the samples into the array of release media where they were soaked and reciprocated. After a specified period of time, the peristaltic pump withdrew a sample from each test tube and transferred it to the VK-8000 auto sampler where pre-capped HPLC vials were placed. When samples were taken from each test tube at the end of each sampling interval, the carriage lifted the samples out of the current tubes and moved them to next row of test tubes for the next interval, where another cycle was performed. In this study, Drug A was released in modified artificial gastric fluid (AGF) media at 2 h per interval with 30 dippings per minute (DPM) for a total of 24 h, while Drug B was released in pH 3 phosphoric acid media at 2 h per interval for a total of 20 h with the same DPM. The designed drug release profiles are ascending profiles for Drug A but zero order profiles for Drug B.

2.3.2. HPLC analysis

The high-performance chromatographic separations were carried out on an Agilent 1100 HPLC system on a 5 μm BDS Hypersil C18 (50 mm × 4.6 mm) column. In the case study of Drug A, the mobile phase composition was pH 3.3 ammonium formate buffer/acetonitrile/methanol, 75/8/17 (v/v/v). Before use, the mobile phase was filtered through a membrane filter (pore size: 0.22 μm) and degassed. Separation was performed at 35 °C, the injected volume was 25 μL, the flow rate was 1.5 mL/min, and the compound A was detected at 275 nm. The HPLC analyses of Drug B release rate samples were performed at 30 °C with a flow rate of 1.0 mL/min using 0.1% phosphoric acid/ACN/MeOH, 74/13/13 (v/v/v) as mobile phase. The injection volume was 20 μL, and drug B was detected at 266 nm. Data acquisition and analysis were performed using Waters Empower software (Build 1154, Waters Corporation, Milford, MA). The two HPLC methods were directly adapted from the existing in-house validated HPLC methods of the products, and were not optimized for fast separation.

2.3.3. μPLC analysis

Quantitative analysis of the drug released from OROS® tablets was also completed on a μPLC system. Chromatographic analyses were performed on a Brio cartridge, which incorporated 24 parallel liquid chromatography columns (80 mm × 0.5 mm) packed with 7 μm C18. A 6-needle autosampler designated for a 96-well plate format was used, and the autosampler was capable of dispensing volumes varying from 0.4 to 5.0 μL. In this study, the injection volume was usually set at 4.5 μL unless noted otherwise. A programmed flow rate of 300 μL/min (12.5 μL/min/column) was used. Separation was performed at ambient temperature with a fixed UV detection at 280 nm for Drug A and 254 nm for Drug B. Veloce Analysis software 1.9 was used for data acquisition, while Microsoft Excel 2000 was used for data processing. Table 1 shows the details of chromatographic conditions for the drug release rate study using both HPLC and μPLC. Since μPLC is by all means still a chromatographic technique, all the HPLC separation parameters can be easily adapted into μPLC analysis, i.e., similar mobile phase combination and detection wavelength. The increased organic composition has been used to compensate the decreased flow rate of μPLC because of the reduced column size and pressure limit requirement of μPLC. Additionally, the detection wavelengths used in μPLC analyses for both drugs are slightly different from HPLC analyses due to the fact that the UV detector of μPLC uses filters for the measurement, and the availability of filters limits the selection of UV wavelengths in μPLC. The best available filters to monitor both drugs were chosen in the μPLC study.

2.3.4. Drug release profile determination

In both HPLC and μPLC analyses, the amount of drug released during specified time intervals was quantified by linear

Table 1
Analytical methods for the analyses of drug release samples

	HPLC method	μPLC method
Chromatographic methods for the analysis of Drug A		
Stationary phase	Thermo BDS Hypersil C18, 50 mm × 4.6 mm, 5 μm	Brio 4208 Cartridge, C18, 80 mm × 0.5 mm, 7 μm
Mobile phase	75% Ammonium formate pH 3.3; 25% ACN/MeOH (8/17, v/v)	58% Ammonium formate pH 3.3; 42% ACN/MeOH (8/16, v/v)
Detection (nm)	275	280
Run time (min)	5.5	6.5
Flow rate	1.5 mL/min	12.5 μL/min/column
Retention time (min)	3.8	3.5
Column temperature (°C)	35	Ambient temperature
Injection volume (μL)	25	4.5
Chromatographic methods for the analysis of Drug B		
Stationary phase	Thermo BDS Hypersil C18, 50 mm × 4.6 mm, 5 μm	Brio 4208 Cartridge, C18, 80 mm × 0.5 mm, 7 μm
Mobile phase	74%, 0.1% phosphoric acid; 26% ACN/MeOH (50/50, v/v)	58%, 0.1% phosphoric acid; 42% ACN/MeOH (50/50, v/v)
Detection (nm)	266	254
Run time (min)	4.5	5.0
Flow rate	1.0 mL/min	12.5 μL/min/column
Retention time (min)	3.5	3.5
Column temperature (°C)	30	Ambient temperature
Injection volume (μL)	20	4.5 μL

Table 2
Reproducibility of μPLC analysis

	Peak Area					%R.S.D. across 24 columns[a]
	Column no. 1	Column no. 7	Column no. 14	Column no. 17	Column no. 24	
Run #1	21.58	21.62	21.43	23.03	22.97	5.4
Run #2	20.48	21.01	21.08	22.05	22.22	4.7
Run #3	21.81	20.52	20.96	21.82	22.76	4.9
Run #4	22.40	21.17	20.50	22.49	22.28	4.9
Run #5	21.05	20.45	21.02	21.72	22.73	5.8
%R.S.D. for each column	3.4	2.3	1.6	2.4	1.4	

Chromatographic conditions as stated in Table 1; Sample, 14.81 μg/mL of Drug A standard solution.
[a] %R.S.D. was calculated based on results from all 24 columns, although the peak areas from only 5 randomly selected columns are listed in the table.

regression analysis of peak area from a standard curve containing at least three standard points. Drug release rate profiles were generated by plotting the amount of drug released against the specified time intervals, while drug cumulative release profiles were obtained by plotting the total amount of drug released against the specified time intervals.

3. Results and discussion

3.1. Representative chromatograms and reproducibility study

Representative chromatograms from μPLC analysis are shown in Fig. 2, where an overlay of 24 chromatograms from a single run is demonstrated. Generally speaking, the μPLC system is capable of analyzing 24 different samples simultaneously. However, the samples have to be analyzed under identical chromatographic conditions.

As previously mentioned, the μPLC system (Nanostream, Inc., Pasadena, CA) consists of a Brio cartridge with 24 parallel columns and a detection system with 24 individual UV absorbance flow cells. In addition, the system autosampler is controlled by a syringe pump, which contains only six syringes. Therefore, the 6-channel autosampler has to operate 4 times in order to load 24 samples before a run actually begins. Therefore, generating 1 set of 24 chromatograms involves 24 columns, 24 flow cells, and 4 replicate injections using an autosampler containing a 6-injection-needle syringe.

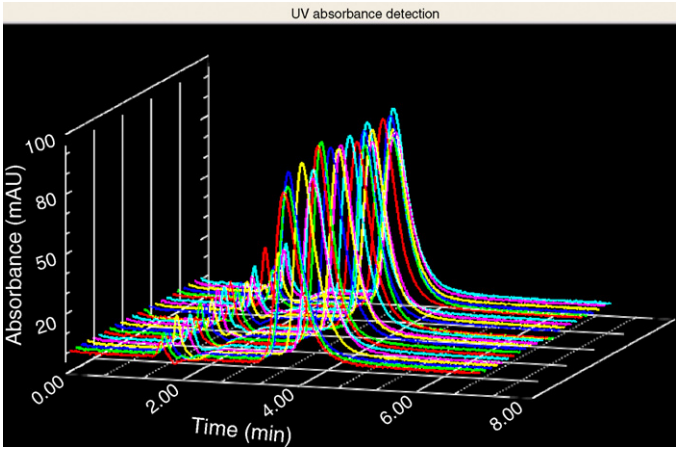

Fig. 2. 3-D view of chromatograms from a single injection on μPLC system.

In this work, the reproducibility of the μPLC was studied by injecting the same sample into all 24 columns. The percent of relative standard deviation (%R.S.D.) of $n = 24$ in terms of peak areas was calculated, and selected results are presented in Table 2. In general, the calculated %R.S.D. of peak area from the 24 columns in μPLC analysis was within 6% at mid-level sample concentration, compared to the normal results of less than 2% for HPLC analysis.

To identify the major factor that contributed to the higher %R.S.D., the reproducibility of the μPLC within the same column was also investigated by injecting the same sample into the same column for five consecutive runs. As seen in Table 2, the %R.S.D. of replicate injections ($n = 5$) of the same sample on an individual column was generally less than 3%, which is much lower than the %R.S.D. of a single injection across all 24 columns. This indicates that the increased %R.S.D. is probably due to the column-to-column variations as well as other variations from injectors and flow cells. As a result, replicate injections were used to generate standard curves in the study. In addition, because of the slightly increased variations in μPLC analyses, μPLC is more suitable for drug release assay in supporting OROS® formulation development and scale up development process.

3.2. Injection accuracy

A syringe pump controls the injection volume of the μPLC system. In this work, injection accuracy was also evaluated on a Brio 4208 with 5 μL injection cartridge. Injection volume was varied from 0.5 to 5.0 μL. Five samples with different concentrations were used for this analysis. The calibration curve was obtained by plotting the peak area against injection volume, as shown in Fig. 3. Good linearity for each sample indicated that accurate injection volumes could be achieved when varied from 0.5 to 5.0 μL.

According to the column dimensions of μPLC, the 5.0 μL injection volume is about 30% of column volume. As known, overloading the column may denigrate the quality of the separation. However, quantitation of the drug is still achievable based on the good linearity result of peak area against injec-

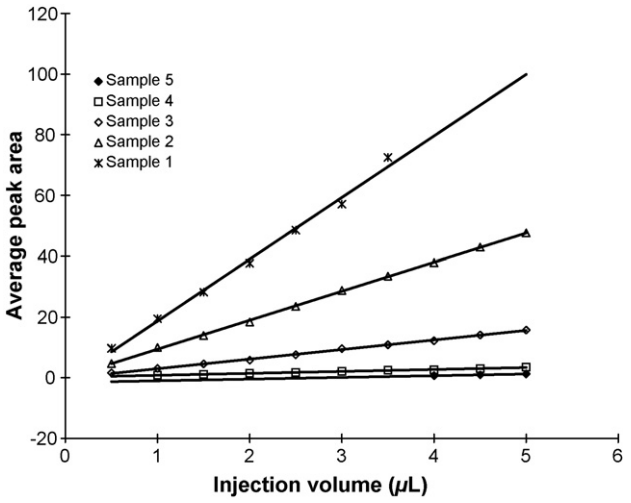

Fig. 3. Effect of injection volume. Results were based on an average of four replicate injections. (*Note:* Only three points in sample 1 were used because others were below the method LOQ. Therefore, they are not included in the figure.).

tion volume. In addition, OROS® delivery technology usually uses many polymers like polyethylene oxide or poloxamer as critical excipients in order to achieve the desired drug release profiles and functionality. Therefore, the drug release samples can become very viscous, which may impact the injection accuracy and reproducibility. Our previous experience indicates that larger injection volumes usually result in better reproducibility and quantitation. Therefore, the injection volume was set at 4.5 μL in this study.

3.3. LOD and LOQ determination

The limit of detection (LOD) and limit of quantification (LOQ) of Drug A were determined using both μPLC and HPLC. It was defined that the %R.S.D. should be less than 20% for six injections for the determination of LOQ. The calculated LOD at (3 × signal-to-noise ratio [*S*/*N*)] for μPLC was 0.5 μg/mL, while the calculated LOQ at (10 × *S*/*N*) was 2.0 μg/mL. These values were about one magnitude higher than those obtained from conventional HPLC (LOD: 0.04 μg/mL, *S*/*N* = 3; LOQ: 0.13 μg/mL, *S*/*N* = 13). The relatively lower sensitivity of μPLC was probably due to the lower signal response, which was caused by the smaller amount of sample injected and the reduced path length of the flow cell in μPLC. The injection volume of μPLC was only 4.5 μL, compared to 25 μL in the HPLC method. The path length of the μPLC flow cell is 2 mm, which is 5 times shorter than that of a standard HPLC flow cell. Additionally, the low sensitivity of μPLC may also result from the lower absorptivity of Drug A at 280 nm, compared to that at 275 nm. In μPLC, the UV detector incorporates an automated filter wheel, which can only hold up to five filters and one blank for dark current measurement. The availability of filters limits the selection of UV wavelengths. Overall, the unique design of the cartridges and instrumentation greatly enhanced the high-throughput capabilities. It can, however, limit sensitivity of analysis.

3.4. Drug release rate study using μPLC

To compare the analysis of release rate samples using both HPLC and μPLC, 2 OROS® tablets, OROS® Push-Pull™ (Drug A) and OROS® Push-Stick™ (Drug B), were used. After the drugs were released from OROS® tablets in the USP Type VII apparatus, at specified time intervals the samples were transferred into both HPLC vials for HPLC analysis and into 96 well plates for μPLC analysis.

The release-rate profiles generated using HPLC and μPLC and the release-rate profiles generated by two analysts using μPLC were compared in Fig. 4. An OROS® Push-Pull™ tablet containing 15 mg of Drug A was used for this study. The two independent analyses gave virtually superimposable drug release profiles, and the release-rate profiles generated by two analysts were also similar. To confirm the equivalence between the two equipments and the two analysts, JMP statistical software (SAS Institute, Cary, NC) was used for statistical analysis. Since the calculated *P* value was less than 0.05, it was considered that the acceptable release-rate difference was small and insignificant between the two equipments and the two analysts.

A similar study was also performed on an OROS® Push-Stick™ formulation (Drug B). Fig. 5 compares drug release profiles generated from HPLC and μPLC. Similar drug release profiles were observed. The data further support that μPLC was capable of analyzing the release rate samples and generating drug release profiles that are superimposable when compared with those from HPLC.

Tables 3 and 4 demonstrate the comparison of total analysis time and total solvent consumption when using HPLC and

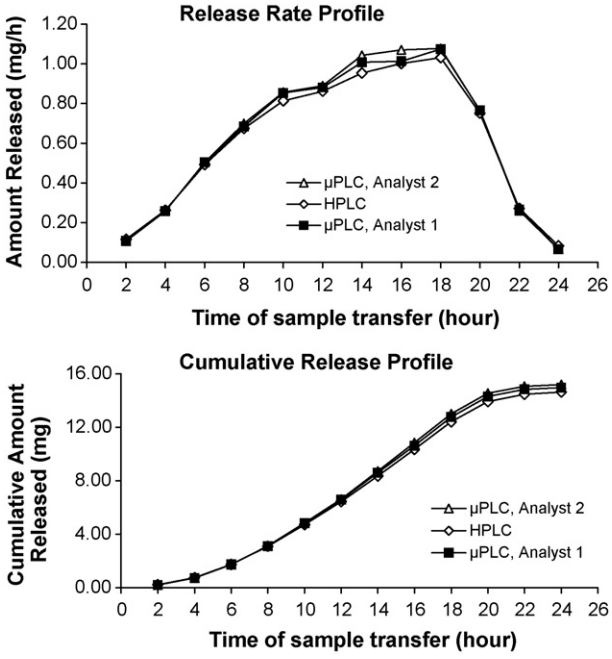

Fig. 4. Comparison of drug release profiles for Drug A. Samples: OROS® Push-Pull™ Drug A with 15 mg dosage; results based on an average of *n* = 12 tablets. Drug release operating parameters: USP Type VII; medium: modified artificial gastric fluid at pH 1.0; bath temperature: 37 °C; agitation rate: 30 dippings per minute; release intervals: 2 h intervals for a duration of 24 h.

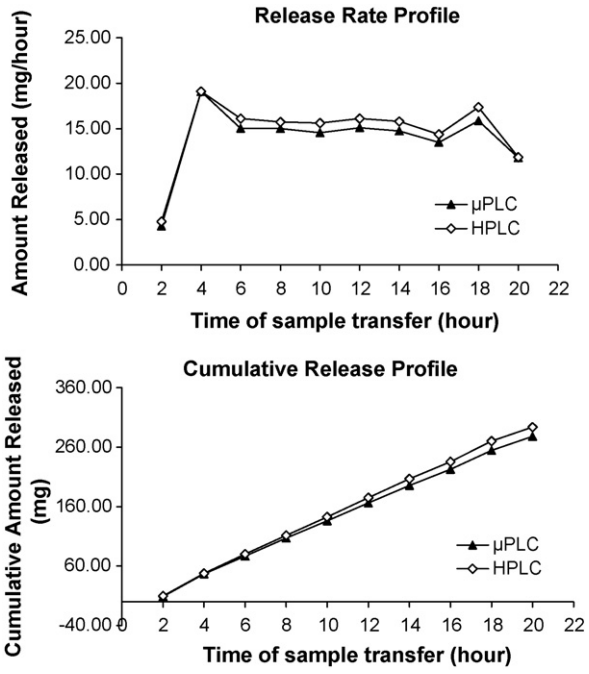

Fig. 5. Comparison of drug release profiles for Drug B. Samples: OROS® Push-Stick™ Drug B with 300-mg dosage; results based on an average of $n = 5$ tablets. Drug release operating parameters: USP Type VII; medium: pH 3 phosphoric acid; bath temperature: 37 °C; agitation rate: 30 dippings per minute; release intervals: 2 h intervals for a duration of 20 h.

Table 3
Comparison of total analysis time

	HPLC method	μPLC method
Drug release (h)	24	24
Sample transfer (min)	5	20
Analysis time (h)	18	2
Data analysis time	2 h	45 min
Total sample analysis time (h)	20	3
Total time reduction	–	~7-fold

Note: Results were based on the Drug A release rate study of 12 tablets × 12 intervals.

Table 4
Comparison of total solvent consumption

	HPLC method	μPLC method
Flow rate	1.5 mL/min	300 μL/min total; 12.5 μL/min/column
Run time (min)	5.5	5.5
Total solvent consumption	1.6 L	36 mL
Total solvent reduction	–	~45-fold

Note: Results were based on Drug A release rate study of 12 tablets × 12 intervals.

μPLC to analyze release rate samples. Analysis of 144 samples (12 tablets × 12 intervals) using μPLC was accomplished in 3 h compared to 20 h by HPLC analysis. In addition, the μPLC system consumed only 36 mL of mobile phase over the course of the study compared to 1.6 L of mobile phase using conventional HPLC. Overall, compared to conventional HPLC, significant reduction in analysis time and solvent consumption were the major advantages of μPLC.

4. Conclusion

The application of μPLC for the analysis of release-rate samples was evaluated for two OROS® formulations. Due to the inherent design of the instrument and its cartridges, μPLC has slightly less reproducibility and sensitivity than HPLC. However, the drug release profiles generated from μPLC showed no significant difference from conventional HPLC. Consequently, μPLC has demonstrated the capability for high-throughput analysis of release-rate samples with satisfactory reproducibility and acceptable accuracy. There were two major advantages in using μPLC compared to HPLC methods. First, the sample analysis was completed in less than 3 h and consumed much less solvent. Secondly, the high-throughput approach of μPLC allowed simultaneous generation of standard curves and replicate sample analyses. Overall, μPLC showed a potential use for the fast analysis of release-rate samples in supporting OROS® product development.

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