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Nota applicativa

Streamlining the Analysis of Oral Contraceptives Using the ACQUITY UPLC H-Class System

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Abstract

In this application work, we consolidate two HPLC methods and one GC method for the analysis of desogestrel, ethinyl estradiol, and their related substances to one UPLC method. UPLC method development was required to consolidate the legacy methods. Various column chemistries, gradient profiles, temperatures, and flow rates were explored and will be discussed in this study.

The resulting UPLC method provides a marked reduction in analysis time, improved resolution, and reduced mobile phase consumption. The new method improves laboratory efficiency and productivity, as well as reducing costs for manufacturing facilities.

Benefits

- 88% reduction in run time
- 96% reduction in mobile phase consumption
- Reduced cost for solvent and waste disposal

Introduction

Generic pharmaceutical companies build profitability based on replicating innovator pharmaceutical products by reducing costs. The cost reductions are typically realized by refining synthetic routes, packaging procedures, and drug delivery mechanisms in an effort to provide a cost-effective solution to the customer. Compendial methods provide an inexpensive solution, given their tasks and available resources. Converting methods to more modern analytical technologies, such as UPLC, provides long-term cost reductions, increased throughput, and improved asset utilization that contributes to overall profitability.

Desogestrel and ethinyl estradiol are hormonal contraceptives available as a combination product. The methods existing in literature for the analysis of combination oral contraceptive products do not address advances in the new chemistries and instrumentation.

In this application work, we consolidate two HPLC methods and one GC method for the analysis of desogestrel, ethinyl estradiol, and their related substances to one UPLC method. UPLC method development was required to consolidate the legacy methods. Various column chemistries, gradient profiles, temperatures,

and flow rates were explored and will be discussed in this study.

The resulting UPLC method provides a marked reduction in analysis time, improved resolution, and reduced mobile phase consumption. The new method improves laboratory efficiency and productivity, as well as reducing costs for manufacturing facilities.

Experimental

Solution preparation

Resolution mixture solution with APIs and impurities

Separate stock solutions were prepared by dissolving an accurate amount of the two active pharmaceutical ingredients (API) and their related substances in methanol to make a solution at 0.3 mg/mL concentration. An equal volume of each stock solution was transferred to one vial to prepare a resolution stock solution at 0.03 mg/mL in methanol. The resolution stock solution was then diluted with water to make a working resolution solution with a final concentration of 0.016 mg/mL of each analyte.

Mixture for UPLC analysis	
API	**Related substances (RS)**
A	A-RS 1
	A-RS 2
	A-RS 3
	A-RS 4
	A-RS 5
	A-RS 6
B	B-RS 1
	B-RS 2
	B-RS 3

Sample solutions

Tablets used in this study contained 0.15 mg of desogestrel and 0.03 mg of ethinyl estradiol. Sample solutions were prepared by dissolving 25 tablets in 6 mL of methanol by sonication for 20 minutes. Solutions were then diluted with 4 mL of water, and sonicated for an additional 10 minutes. Concentration of desogestrel and ethinyl estradiol in the sample solution was 0.375 and 0.075 mg/mL, respectively.

HPLC method 1 conditions

System:	Alliance 2695 HPLC
Column:	Synergi C_{18} Hydro-RP 4.6 x 250 mm, 4 µm
Column temp.:	25 °C
Injection volume:	25.0 µL
Flow rate:	2.0 mL/min
Mobile phase A:	42:58 acetonitrile/water
Mobile phase B:	75:25 acetonitrile/water
Separation mode:	Gradient
Wash solvents:	50:50 water/acetonitrile
Detection:	UV, 210 nm

Gradient

Step	Time (min)	Solvent A (%)	Solvent B (%)
1	Initial	100	0
2	15.0	100	0
3	17.0	0	100
4	45.0	0	100
5	45.1	100	0
6	48.1	100	0

HPLC method 2 conditions

System:	Alliance 2695 HPLC
Column:	SPHERISORB ODS-2 4.6 x 250 mm, 3 µm
Column temp.:	25 °C
Injection volume:	20.0 µL
Flow rate:	1.5 mL/min
Mobile phase A:	25:25:50 acetonitrile/ methanol/water
Mobile phase B:	50:50 acetonitrile/ methanol
Separation mode:	Gradient
Wash solvents:	50:50 water/acetonitrile
Detection:	UV, 210 nm FLR, λex 208 nm, λem 310 nm

Gradient

Step	Time (min)	Solvent A (%)	Solvent B (%)
1	Initial	100	0
2	20.0	100	0
3	30.0	0	100
4	45.0	0	100
5	45.1	100	0

Step	Time (min)	Solvent A (%)	Solvent B (%)
6	50.0	100	0

GC method conditions

Column:	Restek RTX-1 15 m x 0.32 mm, coated with G1 film
Detector:	FID, 280 °C
Injection port temp.:	280 °C
Carrier gas:	Nitrogen, 2.5 mL/min
Oven temp.:	140 °C to 240°C at 10 °C/min, hold at 240 °C for 30 minutes, increase to 300 °C, and hold for 10 minutes, equilibrate to the initial conditions for five minutes

UPLC conditions

System:	ACQUITY UPLC H-Class with PDA and FLR detectors
Column:	ACQUITY UPLC HSS T3 150 x 2.1 mm, 1.8 µm
Column temp.:	67 °C
Sample temp.:	20 °C
Injection volume:	5.0 µL
Flow rate:	0.4 mL/min

Mobile phase A:	100% water
Mobile phase B:	100% acetonitrile
Separation mode:	Gradient

Gradient

Step	Time (min)	Solvent A (%)	Solvent B (%)
1	Initial	67	33
2	10.0	14	86
3	13.5	14	86
4	13.6	67	33
5	18.6	67	33

Results and Discussion

The HPLC methods for analysis of desogestrel, ethinyl estradiol, and their related substances were run as described on the Alliance HPLC System. The GC method was not tested. The breakdown of the legacy methods and the analytical focus is summarized in Table 1.

Components	Legacy methods	UPLC method
API: A	HPLC method 1	ONE method for APIs and related substances
A-RS 1		
A-RS 2		
A-RS 3		
A-RS 4		
API: B		
B-RS 1		
B-RS 2		
A-RS 5	HPLC method 2	
A-RS 6		
B-RS 3	GC	

Table 1. Table summarizing the relationship of analytical technique and their respective analytical focus.

The first HPLC method was used for analysis of six related substances of desogestrel and ethinyl estradiol. This method did not provide adequate sensitivity for some related substances. Therefore, a second HPLC method was developed for the quantification of additional related substances (A-RS 5 and A-RS 6). The HPLC data are shown in Figures 1A and 1B.

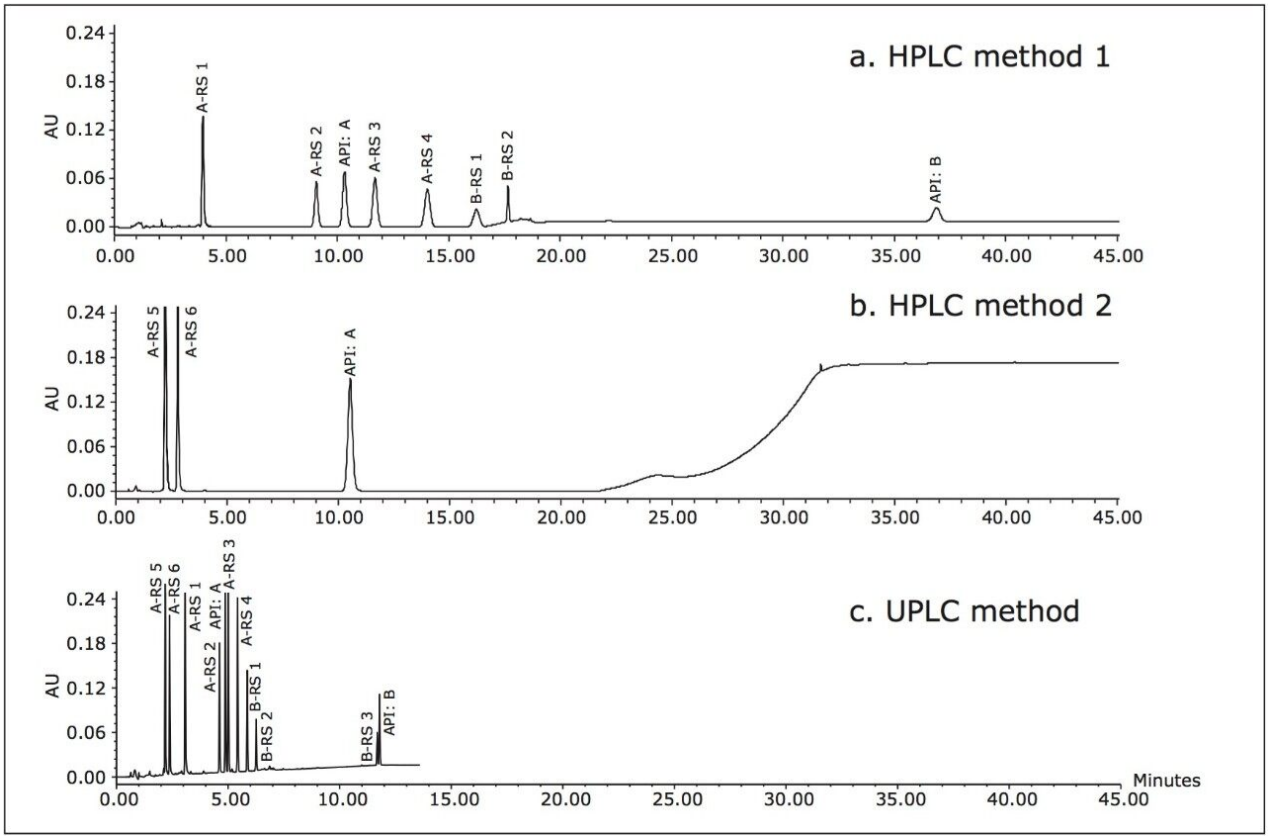


Figure 1. Comparative results of the two HPLC methods performed on the Alliance 2695 System, and the UPLC method performed on the ACQUITY UPLC H-Class System. UV at 210 nm. a. HPLC method 1 (Alliance 2695) b. HPLC method 2 (Alliance 2695) c. UPLC method for separation of all related substances and APIs

The HPLC methods were converted and combined as a single UPLC method using the ACQUITY UPLC H-Class System. The transfer of the HPLC methods to UPLC did not yield the desired resolution of all peaks, largely due to the differences in column selectivity between the existing HPLC methods and available UPLC chemistries. Due to the complexity of combining three analytical approaches, we followed a method development approach to resolve the constituents with a goal of achieving a resolution >1.5 for all peaks.

The UPLC method development was conducted using water (solvent A) and acetonitrile (solvent B), due to the neutral characteristics of the analytes. Different stationary phases were explored such as ACQUITY UPLC BEH, HSS T3, BEH Phenyl, and BEH Shield. The ACQUITY UPLC HSS T3 Column provided the best resolution between the critical pair (B-RS 3 and API: B). Temperatures, various gradient elution conditions, and flow rates were also explored to further optimize separation between all the peaks. A decrease in temperature greater than 20 °C resulted in a coelution of A-RS 4 and B-RS 1. Increasing the gradient slope by increasing the % organic at the end of the gradient resulted in a coelution of B-RS 3 and API: B.

The resulting UPLC method was developed on the ACQUITY UPLC H-Class System using the ACQUITY UPLC HSS T3 150 x 2.1 mm, 1.8 µm Column to successfully resolve all the peaks. The UPLC method is displayed in Figure 1C.

We began our study by reproducing the legacy HPLC methods. As shown in Figures 1A and 1B, the methods provided acceptable resolution of each component; however, the total analysis time for these two methods was 98 minutes. This time increased when factoring in the need for an additional GC analysis (not shown).

We consolidated the methods into a single UPLC method for all the APIs and their related substances, as shown in Figure 1C. By making this transfer, we reduced the analysis time to 18.6 minutes, which represents an overall time savings of 88% and a solvent savings of 96%.

UPLC method

The UPLC method successfully separated the two APIs and their related substances, as shown in Figure 2. The performance of the method was measured by evaluating system suitability of the five replicate injections of the working resolution solution against the system suitability recommendation specified in the USP General Chapter, <621> Chromatography.[2] The UPLC system suitability results for each component are shown in Table 2. The retention times and area repeatability were well below the USP specification of 2% RSD for data from five replicate injections. The resolution between all of the peaks was ≥1.5.

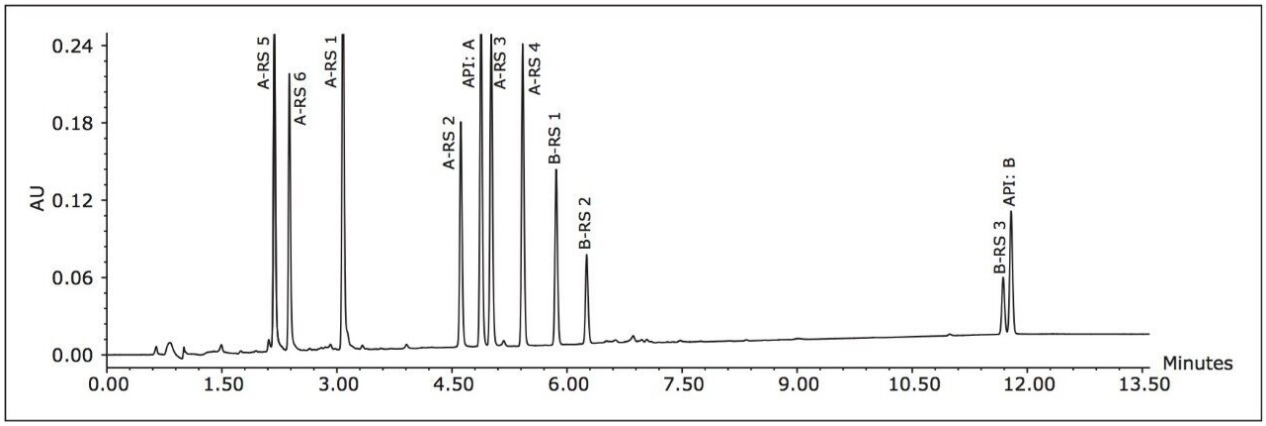


Figure 2. UPLC method with UV at 210 nm. Working resolution solution of API's and related substances performed on the ACQUITY UPLC H-Class System using an ACQUITY UPLC HSS T3 150 x 2.1 mm, 1.8 µm Column.

Component	% RSD RT	% RSD peak areas	USP resolution	USP peak tailing
A-RS 5	0.2	0.3	N/A	1.2
A-RS 6	0.2	0.3	4.0	1.2
A-RS 1	0.2	0.3	13.9	1.2
A-RS 2	0.1	1.1	28.2	1.1
API: A	0.1	0.3	4.5	1.1
A-RS 3	0.1	0.3	2.2	1.1
A-RS 4	0.1	0.4	6.9	1.1
B-RS 1	0.1	0.3	7.1	1.1
B-RS 2	0.1	0.2	6.3	1.1
B-RS 3	0.0	0.4	82.7	1.1
API: B	0.0	0.3	1.5	1.1

Table 2. System suitability results for five replicate injections of the resolution mixture on the ACQUITY UPLC H-Class System

The UPLC method was used for the analysis of commercially available desogestrel and ethinyl estradiol tablets to demonstrate applicability of the method in release testing. T he UPLC data acquired using UV at 210 nm is displayed in Figure 3. Due to the sensitivity challenge with UV, we explored using the FLR detector, as shown in Figure 4. Two related substances, A-RS 5 and A-RS 6, were detected by FLR. A summary of the results for analysis of the drug tablet solution is shown in Table 3.

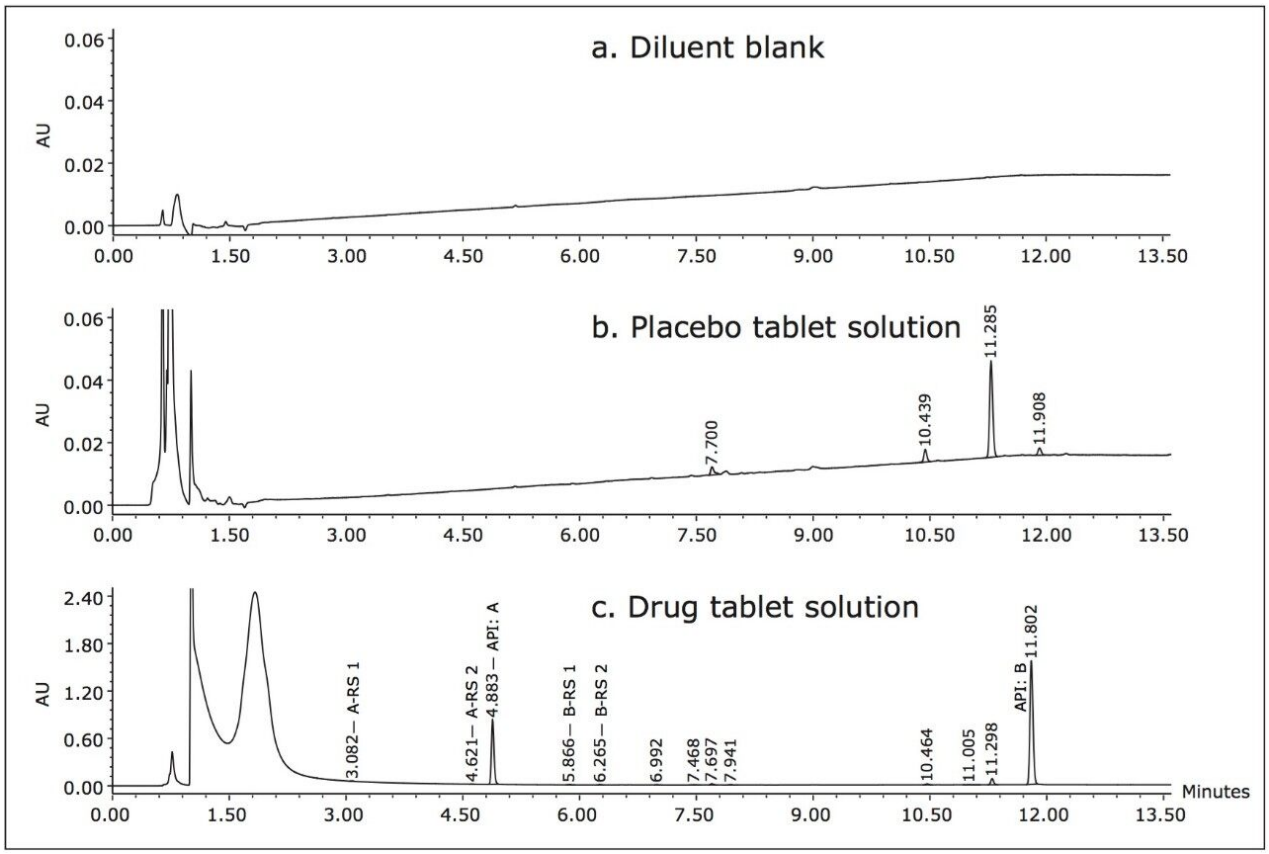
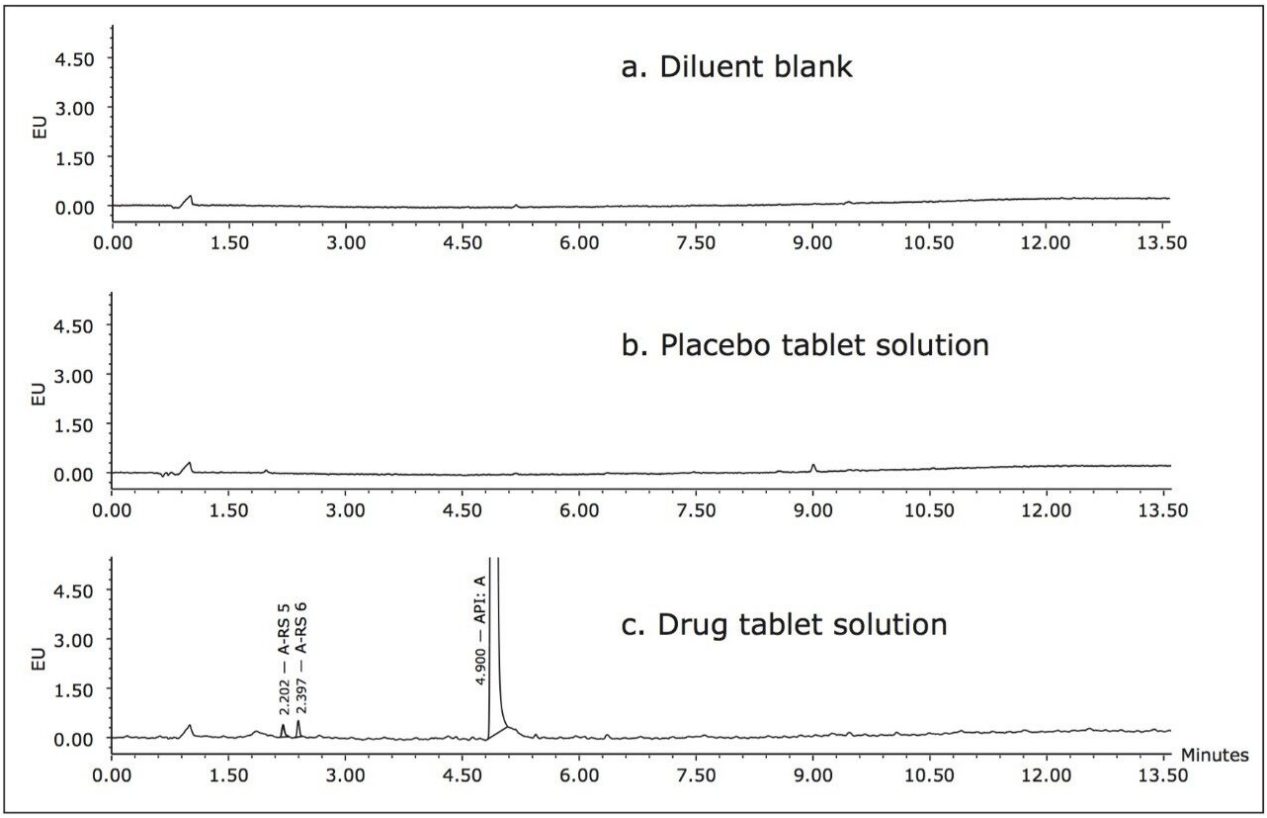


Figure 3. UPLC method with UV at 210 nm. Sample solutions analysis performed on the ACQUITY UPLC H-Class System using an ACQUITY UPLC HSS T3 150 x 2.1 mm, 1.8 µm Column.


a. Diluent blank
b. Placebo tablet solution
c. Drug tablet solution
EU
4.50
3.00
1.50
0.00
0.00
1.50
3.00
4.50
6.00
7.50
9.00
10.50
12.00
13.50
2.202 — A-RS 5
2.397 — A-RS 6
4.900 — API: A
Minutes

Analysis	Component	RT Ratio	% Area*
UV 210 nm	A-RS 5 A-RS 6	None detected	
	A-RS 1	0.63	0.3
	A-RS 2	0.95	0.2
	API: A	1.00	N/A
	A-RS 3 A-RS 4	None detected	
	B-RS 1	0.50	0.3
	B-RS 2	0.53	0.3
	B-RS 3	None detected	
	API: B	1.00	N/A
FLR	A-RS 5	0.45	0.1
	A-RS 6	0.49	0.1
	API: A	1.00	N/A

** % Area reported in the table reflects the peak area of the related substance to the peak area of the corresponding API.*

Table 3. UPLC results for the drug tablet sample solution performed on the ACQUITY UPLC H-Class System using an ACQUITY UPLC HSS T3 150 x 2.1 mm, 1.8 µm Column. UV at 210 nm and FLR detector.

Conclusion

Two HPLC methods and one GC method for analyses of desogestrel, ethinyl estradiol, and related substances were successfully transferred to one UPLC method. The final method maintained the specification for resolution of ≥1.5 between all known peaks. The consolidation of the methodologies eliminates the need for a GC instrument, thus eliminating the cost of operation associated with regular use and maintenance.

The resulting UPLC method provides 88% reduction in run time compared to the three original methods (two HPLC and one GC). The amount of mobile phase used per UPLC injection is 7.44 mL compared to the total of 171.2 mL for two HPLC methods, reducing solvent consumption by 96%.

Implementing UPLC technologies within quality control facilities reduces overall costs, enhances laboratory

throughput and productivity by reducing analysis time for release testing of finished products.

References

1. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721en. 2010 Sept.
2. USP General Chapter, <621> Chromatography, USP35-NF30, The United States Pharmacopeia Convention, official December 1, 2012

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