

UHPLC Method Development for Simultaneous Determination of Antihypertensive Combination Agents

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Key Words

Angiotensin II Receptor Blocker, API, Automated Method Scouting, Autosampler, Diuretics, Generic Method, UHPLC Instrumentation, Vanquish UHPLC System

Goal

To develop an easy to use UHPLC method for the fast separation of combinations of possible antihypertension agents



Introduction

For antihypertension therapy, drug combinations are considered to be a better method to manage and reduce blood pressure than a single drug medication. Therefore, a current trend is the combination of several antihypertension agents in a single pill. Additionally, a single dose application improves the compliance due to the more convenient option of once daily dosing. Typical combinations include agents from different pharmacologic classes, e.g. angiotensin II inhibitors, calcium channel blockers, or thiazides.¹

The goal of this UHPLC method development was a fast separation of potential combinations of antihypertension agents. The latest generation of UHPLC instrumentation² was applied to separate a mixture of one calcium channel blocker (amlodipine besylate), two thiazide diuretics (hydrochlorothiazide and chlorthalidone), and three angiotensin II receptor antagonists (azilsartan, telmisartan, valsartan). Scouting the effect of different mobile phase compositions on the chromatographic result was supported by tools embedded within Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software version 7.2. For subsequent evaluation of best UHPLC method conditions, the report template of the Automated Method Scouting solution was applied (see the related Thermo Scientific Technical Note 161).³ Finally, the method was accelerated by applying the method transfer tool of Chromeleon CDS 7.2.⁴ Due to the characteristics of the 1.5 μm solid core particle material, the separation acceleration did not compromise peak resolution. With this developed UHPLC method, six antihypertension agents are separated within one minute, at a maximum system pressure of 1300 bar (19000 psi).

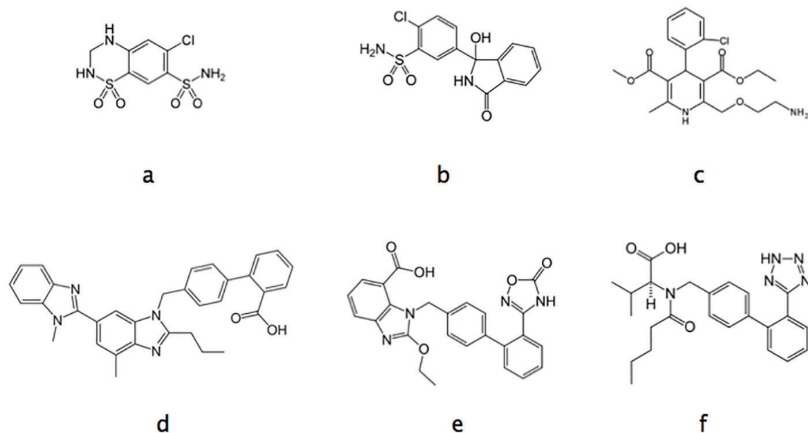


Figure 1. Separated antihypertensive agents: hydrochlorothiazide (a), chlorthalidone (b), amlodipine besylate (c), telmisartan (d), azilsartan (e), valsartan (f).

Equipment

- Thermo Scientific™ Vanquish™ UHPLC system consisting of:
 - System Base (P/N VH-S01-A)
 - Binary Pump H (P/N VH-P10-A)
 - Split Sampler HT (P/N VH-A10-A)
 - Column Compartment H (P/N VH-C10-A)
 - Diode Array Detector HL (P/N VH-D10-A)
- Chromeleon CDS 7.2

Reagents and Chemicals

Compound	Supplier	P/N
Valsartan	Sigma-Aldrich	SML0142-10MG
Azilsartan	Sigma-Aldrich	SML0432-10MG
Telmisartan	Sigma-Aldrich	T8949-10MG
Amlodipine besylate	Sigma-Aldrich	A5605-10MG
Hydrochlorothiazide	Sigma-Aldrich	H4759-5G
Chlorthalidone	Molekula	38140528
Formic acid	Fisher Scientific	A117-50
Ammonium acetate	Fisher Scientific	A114-50
Acetic acid	Fisher Scientific	A113-50
Methanol OPTIMA™ LC/MS	Fisher Scientific	A456-212
Acetonitrile OPTIMA LC/MS	Fisher Scientific	A955-212
Ultra-pure lab water, 18.2 MΩ·cm at 25 °C	n.a.	n.a.

Experimental Conditions

Column:	Thermo Scientific™ Accucore™ Vanquish™ C18+, 1.5 μm, 2.1 × 100 mm (P/N 27101-102130)
Mobile phase:	A1 – Water A2 – Water with 0.1% formic acid A3 – 10 mM ammonium acetate in water, pH 4.5 with acetic acid B1 – Acetonitrile B2 – Acetonitrile with 0.1% formic acid B3 – Methanol
Gradient:	0-1.25 min 5%-90% B, 1.25-2.80 min 90% B, 2.80-3.00 min 90%-5% B, 3.00-5.00 min 5% B
Accelerated gradient:	0-0.625 min 5%-90% B, 0.625-1.40 min 90% B, 1.40-1.50 min 90%-5% B, 1.50-2.50 min 5% B
Flow rate:	0.400 mL/min or 0.800 mL/min (accelerated)
Pressure:	690 bar (max.), 1290 bar (accelerated)
Temperature:	50 °C, still air, easy mode
Injection volume:	1 μL
Sample compartment temperature:	10 °C
Detection:	272 nm, 214 nm, 260 nm (for evaluation), 280 nm, 100 Hz, 0.04 s response time, 4 nm slit width, 4 nm bandwidth, 3D-field: 190-310 nm, 4 nm bandwidth
Flow cell:	LightPipe™, 10 mm (P/N 6083.0100)

Standard Preparation

Single stock solutions of all active pharmaceutical ingredients (APIs) were prepared by solving appropriate amounts in methanol. The stock concentration of amlodipine besylate, valsartan, and azilsartan had a concentration of 2 mg/mL. The stock solution of hydrochlorothiazide had a concentration of 2.60 mg/mL, the stock solution of chlorthalidone had a concentration of 1.81 mg/mL, and the stock solution of telmisartan had a concentration of 1 mg/mL. The single standard solutions were mixed and diluted with water to yield standard mixture containing 320 μg/mL of valsartan, azilsartan, and telmisartan, 40 μg/mL amlodipine besylate, 50 μg/mL chlorthalidone and 50 μg/mL hydrochlorothiazide. The concentrations of the components refer to their typical ratio in hypertension drug formulations.

Results and Discussion

The goal of this application is a fast separation of six hypertensive agents. The developed UHPLC method can be used as a generic method for screening purposes. To achieve sufficient peak capacity at a moderate back pressure, a 2.1×100 mm column with $1.5 \mu\text{m}$ solid core material was chosen. The Vanquish Binary Pump H includes two solvent selectors, each having three fluidic channels. Thereby, the opportunity to choose between nine different solvent combinations is offered by the pump. Automated method scouting becomes applicable without any modification of the system. During this UHPLC method development, five different solvent combinations have been tested. The same generic mobile phase gradient was used for each combination. The organic content started at 5% and was raised to 90% within 1.25 minutes. Furthermore, the optimum flow rate of the column, 0.4 mL/min , was chosen. The scouting of the different solvent combinations was easily achieved by automatically switching the solvent selector valve from run to run. Figure 2 shows the resulting chromatograms.

To find the best method conditions, a Chromeleon CDS assisted workflow which is described in the Technical Note 161³ was applied. Figure 3 shows a run time and resolution map for the sample runs. The shorter the elution time of the last peak, the lower is the bubble on the Y-axis. The diameter of the bubble corresponds to the peak resolution of the critical peak pair. The bigger the bubble, the higher is the resolution. In Figure 3, the bubble on the left related to sample number 18 reveals a good resolution of peaks (large diameter) at a retention time below 2.0 minutes. Therefore, with the generic mobile phase gradient, the best chromatographic result was achieved when using 0.1% formic acid in water combined with 0.1% formic acid in acetonitrile (Sample Number 18 in Figure 3 and Table 1). The excellent retention time reproducibility allows for reliable peak assignments by injecting single standard solutions.

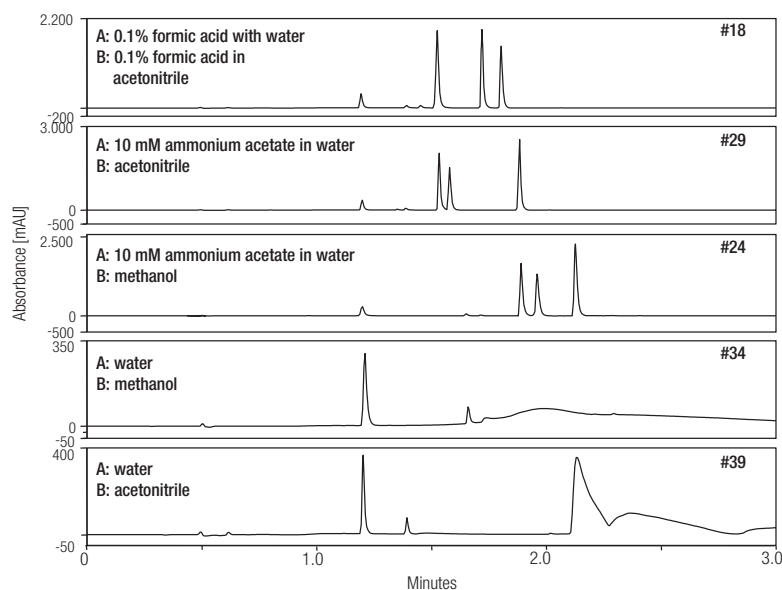


Figure 2. Five different solvent combinations were tested to find the best UHPLC method conditions to separate the standard mixture of antihypertension drugs.

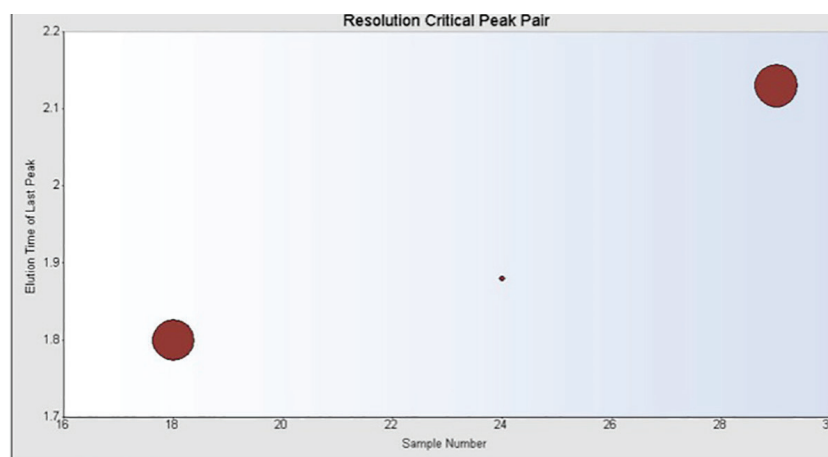


Figure 3. Bubble chart to find the best method conditions, part of the Automated Method Scouting Solution eWorkflow.³

Table 1. Best method conditions, suggested by Chromeleon CDS 7.2 (via Automated Method Scouting report).³

Method Development Sample Number	Eluent Combination		Elution Time of Last Peak [min]	Resolution Critical Peak Pair
18	A2 – 0.1% formic acid with water	B2 – 0.1% formic acid in acetonitrile	1.80	3.10
29	A3 – 10 mM ammonium acetate in water	B3 – methanol	2.13	3.13
24	A3 – 10 mM ammonium acetate in water	B1 – acetonitrile	1.88	2.14
34	A1 – water	B3 – methanol	N/A	N/A
24	A1 – water	B1 – acetonitrile	N/A	N/A

Fused core particle columns are ideally suited for method speed-up as their separation power remains nearly constant when the flow rate is increased.⁵ In addition, the generated back pressure is lower than with porous particle columns. For method speed-up, the original flow rate was doubled and, simultaneously, the mobile phase gradient was adjusted by using the UHPLC method speed-up tool embedded in Chromeleon CDS 7.2. Figure 4 shows an overlay of two chromatograms. Both chromatograms were received with the best solvent combination (Table 1, #18) but either with slow flow rate operating below 700 bar and generic mobile phase gradient (blue line) or doubled flow rate operating near 1300 bar and adjusted mobile phase gradient (black line). The peak evaluation shows no loss in chromatographic performance indicators (see Table 2). However, an improvement was observed as a peak of an unknown impurity was detected after the method speed-up. The unknown impurity peak was clearly separated from the main peak when as less as 10 nL of standard mixture was injected (Figure 5, peak 4).

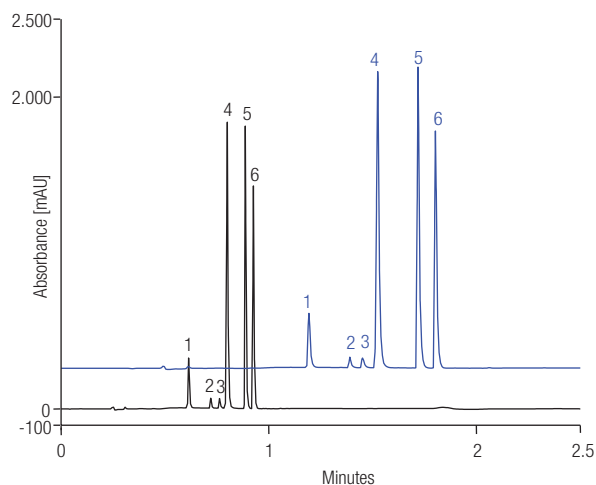


Figure 4. Separation of an antihypertension combination consisting of hydrochlorothiazide (1), chlorthalidone (2), amlodipine (3), telmisartan (4), azilsartan (5) and valsartan (6): The standard mixture was separated at optimum (blue) and twice the optimum flow rate (black).

Table 2. Chromatographic performance indicators for the accelerated (boost factor, BF = 1) and the original method (BF = 1).

Peak No.	Compound	Retention factor k		Peak width (at 50%) [s]		Asymmetry (EP)		Resolution (EP)	
		BF=1	BF=2	BF=1	BF=2	BF=1	BF=2	BF=1	BF=2
1	Hydrochlorothiazide	1.84	1.92	0.687	0.384	1.56	1.48	10.78	10.69
2	Chlorthalidone	2.31	2.43	0.607	0.321	2.01	1.76	3.10	4.19
3	Amlodipine	2.46	2.63	0.795	0.389	2.02	1.97	3.37	3.49
4	Telmisartan	2.63	2.81	0.747	0.375	1.29	1.28	10.15	8.94
5	Azilsartan	3.09	3.22	0.606	0.313	1.79	1.61	4.88	4.38
6	Valsartan	3.29	3.41	0.609	0.314	1.74	1.57	n.a.	n.a.

A calibration curve was recorded for each API by utilizing the wide injection volume range of the Vanquish UHPLC system. In Figure 5, the impressive area reproducibility with 10 nL injection is shown. From six consecutive 0.01 μL injections, the relative standard deviations in peak area and retention time are listed in Table 3. The achieved relative standard deviation of the peak area is below five percent, an excellent value giving the fact that this result was achieved without changing any settings on the instrument hardware or software. Furthermore, injection volumes from 0.01 μL up to 1 μL of a standard mixture diluted tenfold with water were injected to generate the calibration curve. For the smaller peaks chlorthalidone, amlodipine and the unknown, the calibration curve was calculated from 0.05 μL up to 1 μL . The excellent injection linearity of the Vanquish autosampler resulted in superior calibration coefficients as shown in Table 3 and Figure 6.

Table 3. Reproducibility of peak area and retention time at 0.01 μL . Coefficients for calibration curve from 0.01 μL up to 1 μL .

Peak No.	Compound	Reproducibility at lowest injection volume 0.01 μL		Autosampler linearity by injection volumes 0.01 μL – 1 μL
		Retention time RSD [%] (n=6)	Peak Area RSD [%] n=6	Calibration Coefficient (calibration points)
1	Hydrochlorothiazide	0.07	4.98	0.99992 (6)
2	Chlorthalidone	0.07	3.53	0.99991 (5)
3	Amlodipine	0.04	3.32	0.99967 (5)
4	Unknown	0.05	2.22	0.99671 (5)
5	Telmisartan	0.03	2.53	0.99963 (6)
6	Azilsartan	0.04	1.41	0.99774 (6)
7	Valsartan	0.04	3.05	0.99999 (6)

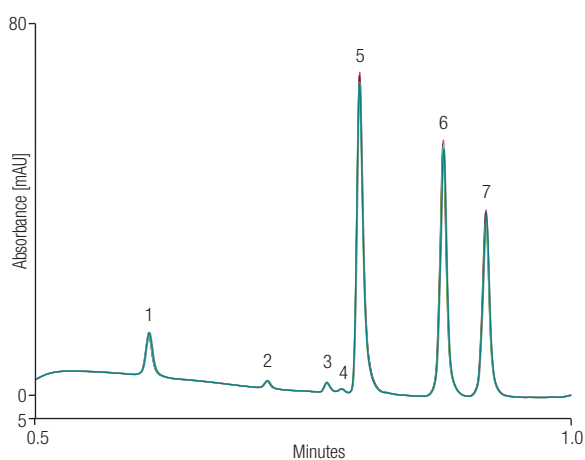


Figure 5. Overlay of six consecutive 0.01 μL injections of standard mixture (after method speed-up).

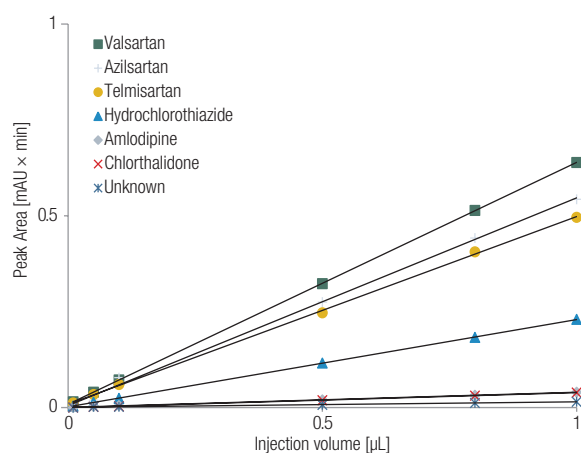


Figure 6. Calibration curves generated for the hypertensive agent mixture (standard mixture diluted tenfold in water) by applying injection volumes from 0.01 μL up to 1 μL .

Conclusion

The fast and easy method development with latest generation UHPLC equipment has been demonstrated. Scouting of five different mobile phase combinations to find the best method condition was done without user interaction. Therefore, the eluent switching capabilities of the Vanquish Binary Pump H and Chromeleon 7.2 software support was applied for data evaluation. The highly efficient Accucore Vanquish C18+ column with 1.5 µm particles allowed method speed-up by a factor of two without compromising resolution. It was demonstrated that the Vanquish Split Sampler HT could reproducibly inject within the nanoliter range and without the need for hardware or software changes. Superior calibration curves were generated by applying the wide injection volume range of the Vanquish Split Sampler HT.

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