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Out-of-the-box usability of Thermo Scientific UltiMate 3000 and Vanquish Core HPLC instruments for the compendial analysis of commonly prescribed drugs

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Keywords

UltiMate 3000, Vanquish Core, USP, EP, Pharmacopeia, lisinopril, omeprazole, amlodipine, metoprolol, hydrochlorothiazide, losartan, gabapentin, furosemide, levothyroxine, method transfer, method lifecycle management

Application benefits

- Demonstration of the suitability of the Thermo Scientific™ UltiMate™ 3000 SD and Thermo Scientific™ Vanquish™ Core HPLC instruments to run compendial methods
- Realization of worry-free setup of compendial workflows by single-sourcing lab equipment and consumables

Goal

This application note is intended to demonstrate the usability of Vanquish Core and UltiMate 3000 HPLC instruments for the straightforward application of compendial methods.

Introduction

Data publicly made available for the year 2019 by the United States Agency for Healthcare Research and Quality[1] show the most prescribed drugs in the U.S. are currently used for continuous treatment of chronic diseases, such as abnormal lipid levels (cholesterol), thyroid hormone deficiency, high blood pressure, type 2 diabetes, breathing problems (asthma, allergies), or seizures, or as diuretic agents (due to heart, liver, or kidney diseases). Exemplarily, the three most often prescribed drugs are

atorvastatin, levothyroxine, and lisinopril with estimated 112, 102, and 92 million annual prescriptions, which, based on daily dosage, corresponds to 7.5%, 6.0%, and 6.1% of all U.S. citizens, respectively.

Many of these drugs have been on the market for several decades, leading not only to a deep understanding of mode of action, potential side-effects, and associated risks, but also to the expiry of the patents and subsequently introduction of drug generics. The resulting price competitiveness is further intensified as these small molecule drugs are often cheap and easy to manufacture. To illustrate, in 2020 off-patent drugs and their generics made up 81.7% of all prescriptions in Germany, while only representing 27.1% of the revenue made with prescription drugs.[2]

An important part of drug manufacturing is the quality control to ensure that the final product meets the requirements with respect to efficacy (drug content) as well as safety (impurities). Standard methods with set requirements, such as those in pharmacopeial monographs created by independent, scientific non-profit organizations, aid the analyst and encourage high quality drug manufacturing. The use of high-performance liquid chromatography (HPLC) for the determination of content and related substances is near-ubiquitous and has been a pillar of drug analysis for several decades. Despite many initiatives in global harmonization as well as modernization of monographs, the lifecycle of these methods often far exceeds the average lifetime of the instruments. This imposes a responsibility on HPLC instrument manufacturers to ensure that technological improvements strike a balance between enabling improvements to chromatography while maintaining the ability to support legacy methods as often encountered in compendial monographs. This is not only true when introducing improvements to an existing product line, but especially important when introducing a new product line.

In this application note, we compare two generations of HPLC instrumentation, namely a Thermo Scientific UltiMate 3000 SD HPLC system and a Thermo Scientific Vanquish Core HPLC system, to demonstrate the usability of these instruments in combination with Thermo Scientific HPLC columns and consumables. Several relevant monographs covering a wide range of chromatographic parameters, retention mechanisms, mobile phase compositions, and additives are selected for this purpose. For this work, we chose the four most common medications for treatment of high blood pressure: lisinopril, amlodipine, metoprolol, and losartan; the by far most important medication for thyroid deficiency: levothyroxine; the dominant medication for seizures: gabapentin; the two most common diuretic agents: furosemide and hydrochlorothiazide; and a common drug for treatment of gastrointestinal problems: omeprazole. With the exception of hydrochlorothiazide and lisinopril, where the official monographs of the European Pharmacopeia (EP) were implemented,[3,4] all procedures were sourced from the United States Pharmacopeia (USP).[5-10]

Experimental

Common experimental details

Chemicals

- Deionized water, 18.2 MΩ·cm resistivity or higher from a Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System (P/N 50136146)
- Acetonitrile Optima™ LC/MS grade (ACN), Fisher Chemical™ (P/N A955-212)
- Methanol Optima™ LC/MS grade (MeOH), Fisher Chemical™ (P/N A456-212)
- Tetrahydrofuran, HPLC grade (THF), Fisher Chemical™ (P/N T425)
- Disodium hydrogen phosphate, anhydrous, ≥99.5%, Fluka™ (P/N 71639)
- Orthophosphoric acid, 85%, HPLC for electrochemical detection, Fisher Chemical™ (P/N 10644732)
- Triethylamine, 99% (TEA), Thermo Scientific™ (P/N 157911000)
- Hydrogen peroxide (30% in water), Fisher BioReagents™ (P/N BP2633-500)
- Sodium dodecyl sulfate, ReagentPlus™ grade, Sigma (P/N L4509-10G)
- 1-Heptanesulfonic acid sodium salt monohydrate, HPLC grade, Thermo Scientific™ (P/N 411270250)
- Sodium hydroxide solution, 50/50 % (w/w), certified, Fisher Chemical™ (P/N SS254-1)
- Sulfamic acid, 99%, Thermo Scientific™ (P/N 222072500)
- Ammonium phosphate, monobasic, Fisher BioReagents™ (P/N BP2427-500)
- Sodium perchlorate monohydrate, >99%, HPLC grade, Fisher Chemical™ (P/N S/5966/50)
- Perchloric acid, 60%, ACS Reagent, Honeywell™ Fluka™ (P/N 311413500ML)
- Acetic acid, Optima™ LC/MS, Fisher Chemical™ (P/N A113-50)
- Sodium phosphate, monobasic, 99%, for analysis, anhydrous, Thermo Scientific™ (P/N 389872500)
- Dibasic sodium phosphate, anhydrous, analytical reagent grade, Fisher Chemical™ (P/N S/4520/53)

Equipment

- Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer (P/N 14-955-152)
- Thermo Scientific™ Orion™ 3 Star pH Benchtop (P/N 13-644-928)
- Vials (amber, 2 mL), Fisher Scientific™ (P/N 03-391-6)
- Cap with Septum (Silicone/PTFE), Fisher Scientific™ (P/N 13-622-292)
- Thermo Scientific™ Finnpipette™ F1 Variable Volume Single-Channel Pipettes

Instrumentation

Thermo Scientific™ Vanquish™ Core system consisting of:

- Vanquish System Base (P/N VH-S01-A-02)
- Vanquish Quaternary Pump C (P/N VC-P20-A)
- Vanquish Split Sampler CT (P/N VC-A12-A)
- Vanquish Column Compartment C (P/N VC-C10-A-03)
- Vanquish Diode Array Detector CG (P/N VC-D11-A)
- Flow Cell path length 10 mm, 13 µL, SST (P/N 6083.0510)
- Vanquish Solvent Monitor (P/N 6230.1310-01)

Thermo Scientific™ UltiMate™ 3000 SD system consisting of:

- Solvent Rack SRD-3400 (P/N 5035.9245)
- Quaternary Pump LPG-3400SD (P/N 5040.0031)
- Sampler WPS-3000 TSL (P/N 5822.0020)
- TCC-3000SD (P/N 730.0010)
- DAD-3000 (P/N 5082.0010)
- Analytical flow cell for DAD-3000(RS) with 10 mm light path, 13 µL, SST (P/N 6082.0100)

Chromatography Data System

The Thermo Scientific™ Chromeleon™ 7.3.1 CDS was used for data acquisition and analysis.

Lisinopril (EP method[3])

Standards

- Ph. Eur. reference standard: Lisinopril dihydrate CRS (P/N EDQM, L0702000)
- Ph. Eur. reference standard: Lisinopril impurity F (P/N EDQM, Y0001234)
- Ph. Eur. reference standard: Lisinopril for peak identification CRS (P/N EDQM, Y0001701)
- Ph. Eur. reference standard: Lisinopril for system suitability A CRS (P/N EDQM Y0001709)

Test for related substances

Sample preparation

Reference solution (a) was prepared accurately weighing 5.0 mg of lisinopril for system suitability A CRS and dissolving in 1.00 mL of mobile phase. A solvent mixture of 50 mL ACN/MeOH (50/50) (v/v) was diluted to 200 mL with an aqueous 3.12 g/L phosphate buffer at pH = 3.2. This mixture was used to dilute 1.00 mL of reference solution (a) to 100 mL to obtain reference solution (b). Reference solution (c) was prepared by dissolving the content of lisinopril impurity F (0.01 mg) in 1.0 mL of mobile phase A. Reference solution (d) was obtained by dissolving 5.0 mg of lisinopril for peak identification CRS in 1.00 mL of mobile phase A. Reference solutions (c) and (d) were used for peak identification purposes only.

Mobile phase preparation

For phosphate buffer 1, 3.12 g of sodium dihydrogen phosphate was accurately weighed and dissolved in 900 mL of ultrapure water. The pH was adjusted to 3.8 using an approximately 10% (m/m) aqueous solution of phosphoric acid. This solution was adjusted to exactly 1,000 mL using ultrapure water. 970 mL of this solution was mixed with 30 mL ACN to obtain mobile phase A. Similarly, for phosphate buffer 2, 3.12 g of sodium dihydrogen phosphate was accurately weighed and dissolved in 900 mL of ultrapure water. The pH was adjusted to 3.5 using an approximately 10% (m/m) aqueous solution of phosphoric acid. This solution was adjusted to exactly 1,000 mL using ultrapure water. 795 mL of this solution was mixed with 205 mL ACN to obtain mobile phase B.

Test for related substances

Chromatographic conditions

Table 1. Chromatographic conditions for lisinopril related substances analysis

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™, 4.6 mm × 250 mm, 5 µm (P/N 25005-254630)
Mobile phase	A: ACN/phosphate buffer 1 (3/97) (v/v) B: ACN/phosphate buffer 2 (20.5/79.5) (v/v)
Gradient	Time (min) %A %B 0.0 100 0 2.0 100 0 37.0 0 100 62.0 0 100 62.1 100 0 75.0 100 0
Flow rate	1.6 mL/min
Column temperature	50°C
Autosampler temperature	10°C
Injection volume	50 µL
Detector settings	210 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Results

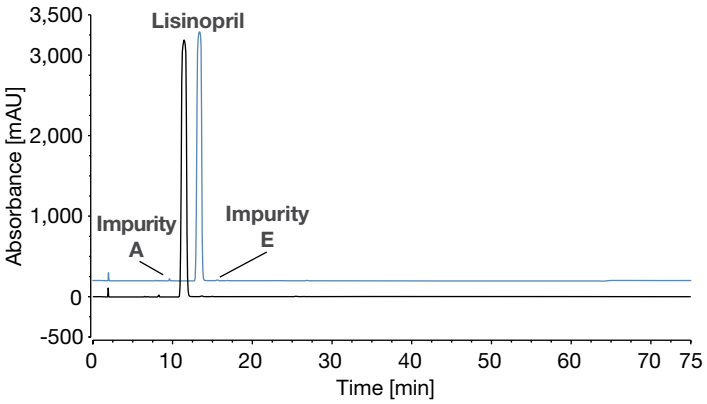

Figure 1. Injections of the lisinopril reference solution (a) on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 2. System suitability requirements for lisinopril analysis

Monograph requirements	Vanquish Core	UltiMate 3000
Resolution (lisinopril, impurity E) >1.5, reference solution (a)	2.74	2.99
Signal to noise ratio >45 for lisinopril, reference solution (b)	115	48

Conclusion

System suitability requirements were met with both instruments.

Table 3. Performance parameters for lisinopril analysis (n=5)

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Impurity A, reference solution (a)	0.72	0.72	0.01	0.29	3.18	2.95	0.36	0.33
Lisinopril, reference solution (a)	1.00	1.00	0.20	0.27	2198.97	2000.82	0.12	0.49
Lisinopril, reference solution (b)	1.00	1.00	0.03	0.03	4.98	4.35	0.41	4.90
Impurity E, reference solution (a)	1.20	1.17	0.01	0.23	2.41	2.40	0.66	0.39

Amlodipine (USP Monograph[4])

Standards

- Amlodipine Besylate certified reference material, Sigma-Aldrich (P/N PHR1185)

Assay

Sample preparation

For the standard preparation, 3.0 mg of USP Amlodipine Besylate RS was accurately weighed and dissolved in 10.0 mL mobile phase. 166 µL of this solution was further diluted in 1.00 mL to obtain a solution with a known concentration of 0.05 mg/mL.

Mobile phase preparation

Phosphoric acid was added to a 7.0 mL solution of triethylamine in about 700 mL of ultrapure water until a final pH of 3.0 was reached. Afterwards, the solution was diluted with ultrapure water to 1,000 mL. To 500 mL of this solution, 350 mL MeOH and 150 mL ACN were added and thoroughly mixed.

Chromatographic conditions

The chromatographic conditions from the monograph were translated according to the guidance outlined in the General Chapter <621> of the USP to adapt for the different inner diameter of the used column (4.6 mm inner diameter versus a prescribed column of 3.9 mm inner diameter).

Table 4. Chromatographic conditions for amlodipine assay and test for related substances

Parameter	Value
Column	Hypersil GOLD, 4.6 mm × 150 mm, 5 µm (P/N 25005-154630)
Mobile phase	Aqueous TEA buffer, pH 3/MeOH/ACN (50/35/15) (v/v/v)
Flow rate	1.39 mL/min
Column temperature	25°C
Autosampler temperature	10°C
Injection volume	13.9 µL
Detector settings	237 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Test for related substances

Sample preparation

The system suitability solution was prepared by dissolving 5.0 mg of amlodipine besylate reference standard in 5.0 mL of hydrogen peroxide followed by heating at 70°C for 45 min. The standard solution was obtained by weighing 1.0 mg of amlodipine and dissolving it in 10.0 mL mobile phase from which 30 µL was further diluted in 1.00 mL to obtain a concentration of 0.003 mg/mL.

Chromatographic conditions

Chromatographic conditions are as given in Table 4 for the assay.

Results

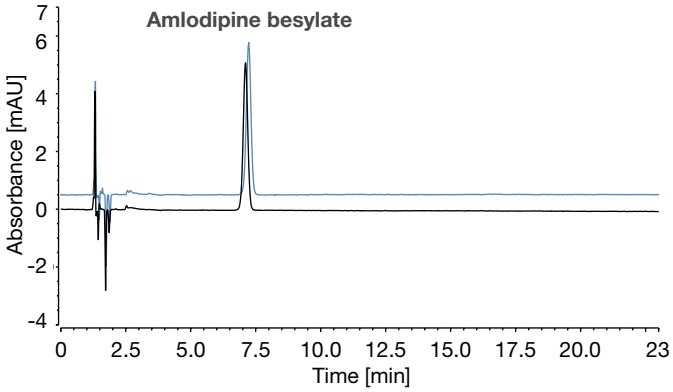

Figure 2. Injection of standard solution for related substances of amlodipine besylate on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 5. System suitability requirements for amlodipine analysis (n=6)

Monograph requirements	Vanquish Core	UltiMate 3000
Assay: RSD NMT 2.0%, standard preparation	0.07%	1.90%
Related substances: resolution (amlodipine impurity A – amlodipine) >4.5, system suitability solution	15.42	15.55
Related substances: RSD NMT 10.0%, amlodipine, standard solution	0.96%	1.01%

Conclusion

System suitability requirements were met with both instruments.

Table 6. Performance parameters for amlodipine analysis (n=6)

	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
Peak	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Amlodipine, assay, standard preparation	1.00	1.00	0.03*	0.06	15.56	13.93	0.07	1.90
Amlodipine, related substances, standard solution	1.00	1.00	0.03	0.02	1.07	1.11	0.96	1.01

* based on standard solution

Metoprolol Tartrate (USP monograph[5])

Standards

- Metoprolol tartrate, certified reference material, Sigma-Aldrich (P/N PHR1076-1G)
- Metoprolol Related Compound A, certified reference material, Sigma-Aldrich (P/N PHR2509-50MG)
- USP Metoprolol Related Compound B, USP (P/N 1441243)
- Metoprolol Related Compound C, certified reference material, Sigma-Aldrich (P/N PHR2510-30MG)
- USP Metoprolol Related Compound D, USP (P/N 1441265)

Assay

Sample preparation

All solutions were used within 48 h of preparation. 25.0 mg of metoprolol tartrate RS was added to a 25.0 mL volumetric flask, which was then accurately filled with mobile phase.

Mobile phase preparation

1.30 g of sodium dodecyl sulphate was dissolved in approximately 600 mL ultrapure water. 100.0 mg of phosphoric acid was added to this solution and then ultrapure water to exactly 1,000 mL. From this solution, 600 mL were mixed with 400 mL of ACN to obtain the mobile phase.

Chromatographic conditions

Table 7. Chromatographic conditions for metoprolol tartrate assay and organic impurities

Parameter	Value
Column	Thermo Scientific™ HyPURITY™ C8, 4.6 mm × 150 mm, 5 µm (P/N 22205-154630)
Mobile phase	Buffer/ACN (60/40) (v/v)
Flow rate	1 mL/min
Column temperature	30°C
Autosampler temperature	10°C
Injection volume	10 µL
Detector settings	223 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Organic impurities

Sample preparation

The system suitability (SST) solution comprising 5 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS was prepared by adding 2.5 mg of each of the three impurities to separate 5.0 mL flasks and accurately filling them with mobile phase. Then, to a 100 mL volumetric flask was added 1.00 mL of each impurity solution and 500 µL of the 1 mg/mL metoprolol solution that was prepared for the assay. The SST solution was then filled to 100 mL with mobile phase and filtered through a 0.45 µm regenerated cellulose syringe filter. The standard solution comprising 1 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Related Compound C RS, and USP Metoprolol Related Compound D RS in mobile phase was prepared by adding 100 µL of the 1 mg/mL metoprolol tartrate solution from the assay and 200 µL of each of the 0.5 mg/mL impurity solutions to a 100 mL volumetric flask and filtered as described before.

Chromatographic conditions

Chromatographic conditions are as given in Table 7 for the assay.

Results

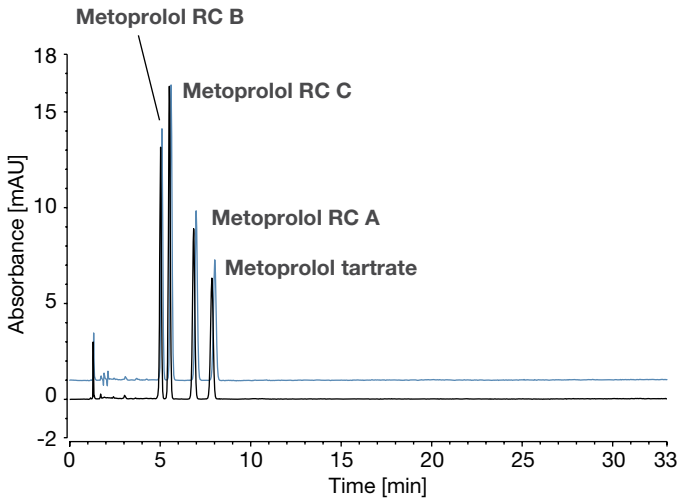

Figure 3. Injections of system suitability solution for metoprolol tartrate impurity method on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 8. System suitability requirements for metoprolol tartrate analysis

Monograph requirement	Vanquish Core	UltiMate 3000
Assay: tailing factor NMT 2	1.15	1.16
Assay: area RSD NMT 0.73%	0.05%	0.08%
Impurities: resolution (metoprolol related compound A – metoprolol related compound B) NLT 1.5	6.32	6.34
Impurities: resolution (metoprolol related compound B – metoprolol related compound C) NLT 2.5	2.68	2.75
Impurities: area RSD NMT 5.0% for metoprolol with standard solution	0.85%	1.34%

Conclusion

System suitability requirements were met with both instruments.

Table 9. Performance parameters for metoprolol tartrate analysis (n=3 for related compounds, n=6 for metoprolol tartrate)

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Metoprolol related compound C	0.64	0.64	0.02	0.04	0.367	0.376	0.17	0.47
Metoprolol related compound B	0.70	0.70	0.02	0.03	0.471	0.456	0.32	0.48
Metoprolol related compound A	0.87	0.87	0.01	0.04	0.307	0.314	0.98	0.94
Metoprolol	1.00	1.00	0.05	0.06	0.276	0.273	0.85	1.34
Metoprolol related compound D	3.56	3.56	-	-	0.390	0.401	5.23	3.15

Losartan (USP monograph[6])

Standards

- Losartan potassium, certified reference material, Sigma-Aldrich (P/N PHR1602-1G)
- Triphenylmethanol, Thermo Scientific™ (P/N A10366)

Assay

Sample preparation

0.1% phosphoric acid in water (solution A) and ACN (solution B) were prepared as mobile phase. Diluent was prepared by mixing 40 mL MeOH and 60 mL ultrapure water. The standard solution was prepared by dissolving 12.5 mg losartan potassium reference standard in 50 mL diluent.

Chromatographic conditions

Table 10. Chromatographic conditions for losartan assay

Parameter	Value
Column	Hypersil GOLD C18 4 mm × 250 mm, 5 µm (P/N 25005-254030)
Mobile phase	0.1% phosphoric acid/ACN (60/40) (v/v)
Flow rate	1 mL/min
Column temperature	35°C
Autosampler temperature	10°C
Injection volume	10 µL
Detector settings	254 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Organic impurities

Sample preparation

The mobile phases were identical to the assay. For the system suitability solution, 5.0 mg triphenylmethanol was dissolved in 25 mL MeOH. 500 µL of the solution was added to 15.0 mg of losartan potassium reference standard and filled to 50 mL with MeOH. The standard solution was prepared by diluting 60 µL of assay standard solution in 50 mL MeOH. The sensitivity solution was prepared by mixing 5.0 mL of standard solution with 5.0 mL of MeOH.

Chromatographic conditions

Chromatographic conditions are as given in Table 11 for the assay.

Table 11. Chromatographic conditions for losartan impurities

Parameter	Value		
Column	Hypersil GOLD C18 4 mm × 250 mm, 5 µm (P/N 25005-254030)		
Mobile phase	A: 0.1% phosphoric acid B: ACN		
Gradient	Time (min)	%A	%B
	0.0	75	25
	25.0	10	25
	35.0	10	90
	45.0	75	25
	50.0	75	25
Flow rate	1 mL/min		
Column temperature	25°C		
Autosampler temperature	10°C		
Injection volume	10 µL		
Detector settings	220 nm, 4 nm bandwidth, 10 Hz, 0.5 s		

Results

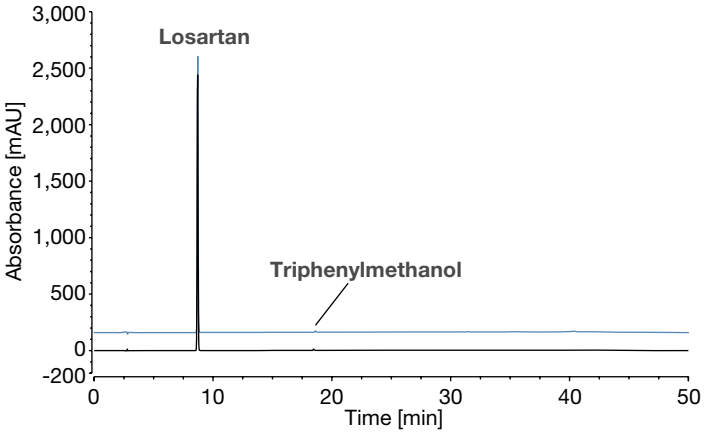

Figure 4. Injections of system suitability solution for impurity method for losartan potassium on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 12. System suitability requirements for losartan potassium analysis

Monograph requirement	Vanquish Core	UltiMate 3000
Assay: area RSD NMT 0.5%	0.05%	0.12%
Assay: tailing factor NMT 1.4	1.01	1.02
Impurities: tailing factor NMT 1.6, system suitability solution	1.01	1.05
Impurities: area RSD NMT 5.0%, standard solution	0.70%	0.68%
Impurities: signal-to-noise NLT 10, sensitivity solution	37.00	35.00

Conclusion

System suitability requirements were met with both instruments.

Table 13. Performance parameters for losartan potassium analysis

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Losartan, assay, standard solution (n=6)	1.00	1.00	0.05	0.07	67.21	67.76	0.05	0.12
Losartan, impurities, standard solution (n=6)	1.00	1.00	0.03	0.02	0.213	0.207	0.70	0.68
Losartan, impurities, system suitability solution (n=3)	1.00	1.00	0.03	0.04	204.4	201.7	0.06	1.05
Triphenylmethanol, impurities, system suitability solution (n=3)	2.12	2.14	0.01	0.01	1.22	1.11	0.11	1.37

Levothyroxine (USP monograph[7])

Standards

- Levothyroxine sodium hydrate, certified reference material, Sigma-Aldrich (P/N PHR2880-100MG)
- Levothyroxine, certified reference material, Sigma-Aldrich (P/N PHR1613-1G)
- Liothyronine, certified reference material, Sigma-Aldrich (P/N PHR1504-500MG)
- USP Levothyroxine Peak Identification Mixture (3 × 5 mL) (1 mg/mL), USP (P/N 1365043)

Assay

Sample preparation

Solution A was prepared by diluting 0.80 mL of sodium hydroxide solution (50% (m/m)) to 500 mL using ultrapure water. A volume of 500 mL MeOH was added. For the levothyroxine stock solution, 40.7 mg levothyroxine was dissolved in 100 mL solution A. The liothyronine stock solution was prepared by dissolving 39.7 mg liothyronine in 100 mL solution A, followed by further diluting 100 µL of this mixture to 10.0 mL with mobile phase.

For the standard solution, 250 µL levothyroxine stock solution and 500 µL liothyronine stock solution were diluted to 10.0 mL using mobile phase.

Mobile phase preparation

For the mobile phase, 800 mL ACN was added to 1,200 mL ultrapure water that contained 1.00 mL of phosphoric acid.

Chromatographic conditions

Table 14. Chromatographic conditions for levothyroxine assay

Parameter	Value
Column	Thermo Scientific™ BetaSil™ Cyano 4.6 mm × 250 mm, 5 µm (P/N 70805-254630)
Mobile phase	0.083% phosphoric acid/ACN (60/40) (v/v)
Flow rate	1.5 mL/min
Column temperature	25°C
Autosampler temperature	10°C
Injection volume	100 µL
Detector settings	225 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Organic impurities, procedure 1

Sample preparation

The diluent consisted of 300 mL ACN added to 300 mL ultrapure water. Solution A consisted of 5.0 mL phosphoric acid diluted to 100 mL with diluent. A 10 N sodium hydroxide solution was prepared by diluting 5.3 mL 50 % (w/w) sodium hydroxide to a total volume of 10.0 mL using ultrapure water. For the levothyroxine stock solution, 25.045 mg levothyroxine was dissolved in 50 mL diluent to which one drop of 10 N sodium hydroxide solution was added with a disposable plastic pipette. This solution was sonicated using an ultrasonic bath for 30 s until fully dissolved. In a next step, 7.0 mL of solution A was added, and the solution was diluted to a final volume of 100 mL using the diluent. The liothyronine stock solution was prepared by weighing 25.0 mg of liothyronine into 50 mL diluent containing 1 drop of 10 N sodium hydroxide, which was added with a disposable plastic pipette. The mixture was dissolved by placing it in an ultrasonic bath for 30 s. Afterwards, 7.0 mL of solution A was added, and the final volume was adjusted to 100 mL with diluent. For the system suitability solution, 5.0 mL of levothyroxine stock solution, 5.0 mL of liothyronine stock solution, and 7.0 mL solution A were combined and diluted to a final volume of 100 mL using diluent. To prepare the standard solution, 4.0 mL of system suitability solution and 7.0 mL of solution A were diluted to a final volume of 100 mL using diluent. For the blank solution, 7.0 mL solution A was diluted to a final volume of 100 mL with diluent.

Mobile phase preparation

An amount of 1.09 g of sodium 1-heptanesulfonate monohydrate was accurately weighed and dissolved in 200 mL water. To this solution, 200 mL of ACN, 400 mL MeOH, and 1.00 mL of phosphoric acid were added. After thorough mixing, the solution was diluted to 1,000 mL using ultrapure water to obtain the mobile phase.

Chromatographic conditions

Table 15. Chromatographic conditions for levothyroxine organic impurities procedure 1

Parameter	Value
Column	Hypersil GOLD C8, 4.6 mm × 150 mm, 5 µm (P/N 25205-154630)
Mobile phase	0.083% phosphoric acid/ACN (60/40) (v/v)
Gradient	Isocratic
Flow rate	1.5 mL/min
Column temperature	35°C
Autosampler temperature	10°C
Injection volume	15 µL
Detector settings	225 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Organic impurities, procedure 2

Sample preparation

For 1,000 mL diluent 1, 900 mL MeOH, and 100 mL solution A were combined. A volume of 1,000 mL diluent 2 was prepared by adding 150 mL ACN to 350 mL solution A and mixing it with 500 mL diluent 1. For the identification solution, 200 µL of levothyroxine peak identification mixture was added to 800 µL diluent 2. Standard stock solution was prepared by dissolving 109.9 mg levothyroxine and 10.1 mg liothyronine in 100 mL diluent 2. By diluting 200 µL of the standard stock solution with diluent 2 to a total volume of 10.0 mL, the standard solution was obtained. Further dilution of 1.00 mL standard solution 1:10 to a total volume of 10.0 mL with diluent 2 resulted in the sensitivity solution. A volume of 1 mL diluent 2 was used as the blank solution.

Mobile phase preparation

A 2 N sodium hydroxide solution was prepared by diluting 528 µL of a 50% (w/w) sodium hydroxide solution to a final volume of 10 mL using ultrapure water. For solution A, 9.77 g sulfamic acid and 3.00 g of a 50% (w/w) sodium hydroxide solution were dissolved in approximately 950 mL of ultrapure water. The pH was then adjusted to 2.0 using a 2 N sodium hydroxide solution before diluting to exactly 1,000 mL. Solution B was 1,000 mL pure ACN.

Chromatographic conditions

Table 16. Chromatographic conditions for levothyroxine organic impurities procedure 2

Parameter	Value		
Column	Hypersil GOLD, 4.0 mm x 150 cm, 3 µm L1, (P/N 25003-154030)		
Mobile phase	A: aq. sulfamic acid solution, pH 2.0 B: ACN		
Gradient	Time (min)	%A	%B
	0	70	30
	10	70	30
	40	20	80
	50	20	80
	53	70	30
	75	70	30
Flow rate	1.0 mL/min		
Column temperature	25°C		
Autosampler temperature	10°C		
Injection volume	25 µL		
Detector settings	225 nm, 4 nm bandwidth, 10 Hz, 0.5 s		

Results

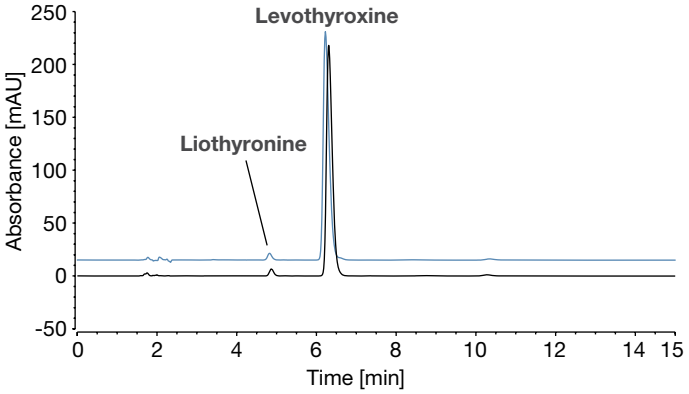

Figure 5. Injections of system suitability solution for the assay of levothyroxine on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

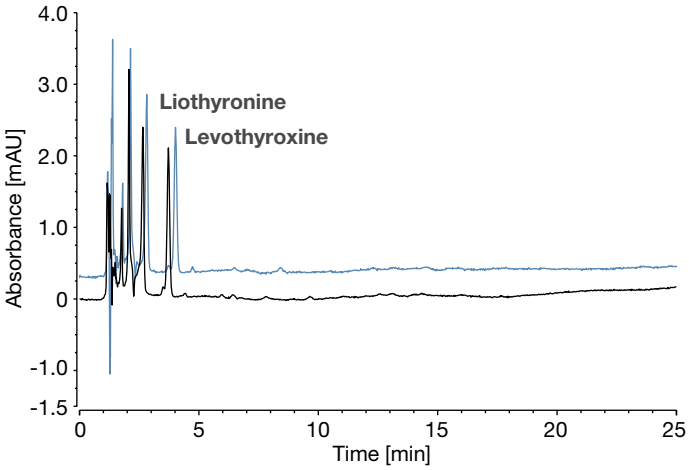

Figure 6. Injections of standard solution for the organic impurities procedure 1 of levothyroxine on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

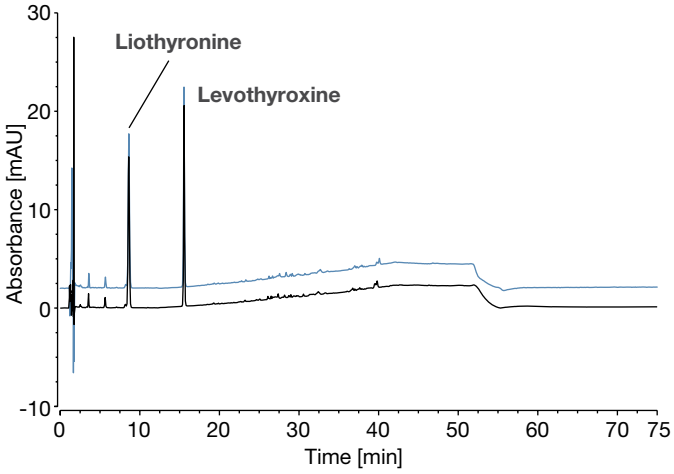

Figure 7. Injections of standard solution for the organic impurities procedure 2 of levothyroxine on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 17. System suitability requirements for levothyroxine analysis

Monograph requirement	Vanquish Core	UltiMate 3000
Assay: resolution (liothyronine, levothyroxine) NLT 5.0, standard solution, n=6	6.19	5.96
Assay: RSD NMT 2.0% for levothyroxine, standard solution, n=6	0.07	1.92
Impurities, procedure 1: resolution (liothyronine, levothyroxine) NLT 5.0, system suitability solution, n=1	5.96	6.21
Impurities, procedure 1: RSD NMT 2.0% for levothyroxine, standard solution, n=6	1.05	0.23
Impurities, procedure 2: resolution (liothyronine, levothyroxine) NLT 5.0, standard solution, n=1	27.9	27.7
Impurities, procedure 2: signal-to-noise ratio for levothyroxine NLT 5, sensitivity solution, n=1	127	103
Impurities, procedure 2: signal-to-noise ratio for liothyronine NLT 5, sensitivity solution, n=1	92	68

Conclusion

System suitability requirements were met with both instruments.

Table 18. Performance parameters for levothyroxine analysis (n=6)

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Levothyroxine, assay, standard solution	1.00	1.00	0.36	0.15	37.002	36.403	0.07	1.92
Liothyronine, assay, standard solution	0.77	0.77	0.33	0.15	0.825	0.869	0.32	3.59
Liothyronine, impurities 1, standard solution	0.71	0.70	0.06	0.09	0.334	0.333	3.20	1.16
Liothyronine, impurities 2, standard solution	0.55	0.55	0.01	0.20	2.920	2.980	0.01	0.05
Levothyroxine, impurities 1, standard solution	1.00	1.00	0.07	0.08	0.253	0.266	1.05	0.23
Levothyroxine, impurities 2, standard solution	1.00	1.00	0.05	0.09	2.650	2.690	0.23	0.09

Gabapentin (USP monograph[8])

Standards

- Gabapentin, certified reference material, Sigma-Aldrich (P/N PHR1049-1G)
- Gabapentin Related Compound A, certified reference material, Sigma-Aldrich (P/N PHR1322-100MG)
- Gabapentin Related Compound B, certified reference material Sigma-Aldrich (P/N PHR2011-100MG)
- Gabapentin Related Compound D, certified reference material Sigma-Aldrich (P/N PHR2012-20MG)
- Gabapentin Related Compound E, certified reference material Sigma Aldrich (P/N PHR2013-50MG)

Assay

Sample preparation

The diluent was prepared by dissolving 0.245 g monobasic ammonium phosphate in 100 mL ultrapure water. The pH of the diluent was adjusted to 2.0 using phosphoric acid. For the standard solution, 70.0 mg gabapentin was dissolved in 5.0 mL diluent. A volume of 164 µL standard solution was added to 836 µL diluent. In a next step, 820 µL of solution was further diluted to 5.0 mL with diluent to obtain the system suitability solution.

Mobile phase preparation

An amount of 0.693 g of monobasic ammonium phosphate and 2.504 g of sodium perchlorate monohydrate were accurately weighed and dissolved in approximately 1,000 mL of ultrapure water. Using perchloric acid, the pH was adjusted to a value of 1.8 and the volume to 1.20 L using ultrapure water. A volume of 760 mL of this buffer solution was mixed with 240 mL of ACN to obtain the mobile phase.

Chromatographic conditions

Table 19. Chromatographic conditions for gabapentin assay

Parameter	Value
Column	Hypersil GOLD, 4.6 mm × 250 mm, 5 µm (P/N 25005-254630)
Mobile phase	Buffer/ACN (76/24) (v/v)
Gradient	Isocratic
Flow rate	1 mL/min
Column temperature	40°C
Autosampler temperature	10°C
Injection volume	20 µL
Detector settings	215 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Early eluting organic impurities

Sample preparation

For the system suitability stock solution, 14.09 mg gabapentin related compound A and 8.24 mg gabapentin related compound B were dissolved in 10.0 mL MeOH. A volume of 50 µL of the system suitability stock solution was diluted with diluent to a total volume of 5.0 mL to obtain the system suitability solution. For the standard solution, an amount of 8.36 mg gabapentin related compound E was dissolved in 10.0 mL diluent. An amount of 70.87 mg of gabapentin was dissolved in approximately 3 mL diluent. To the latter, 50 µL of the gabapentin related compound E solution was added and the final volume was adjusted to 5.0 mL using diluent.

Chromatographic conditions

Table 20. Chromatographic conditions for gabapentin early eluting impurities

Parameter	Value
Column	Hypersil GOLD, 4.6 mm × 250 mm, 5 µm (P/N 25005-254630)
Mobile phase	Buffer/ACN (76/24) (v/v)
Gradient	Isocratic
Flow rate	1 mL/min
Column temperature	40°C
Autosampler temperature	5°C
Injection volume	20 µL
Detector settings	215 nm, 4 nm bandwidth, 10 Hz, 0.5s

Late eluting organic impurities

Sample preparation

An amount of 2.77 mg gabapentin related compound D was dissolved in 2.0 mL of MeOH and diluted to a volume of 10.0 mL using diluent. An aliquot of 100 µL of this solution was further diluted to a final volume of 10.0 mL with diluent to obtain the standard solution.

Mobile phase preparation

Volumes of 350 mL ACN, 300 mL MeOH, and 350 mL buffer were mixed to obtain the mobile phase.

Chromatographic conditions

Table 21. Chromatographic conditions for gabapentin late eluting impurities

Parameter	Value
Column	Hypersil GOLD, 4.6 mm × 250 mm, 5 µm (P/N 25005-254630)
Mobile phase	Buffer/ACN/MeOH (35/35/30) (v/v/v)
Gradient	Isocratic
Flow rate	1 mL/min
Column temperature	40°C
Autosampler temperature	10°C
Injection volume	20 µL
Detector settings	215 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Results

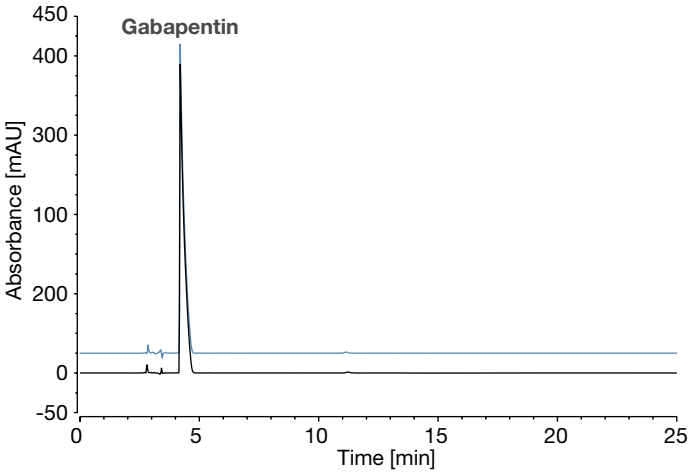

Figure 8. Injections of gabapentin standard solution for the assay on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

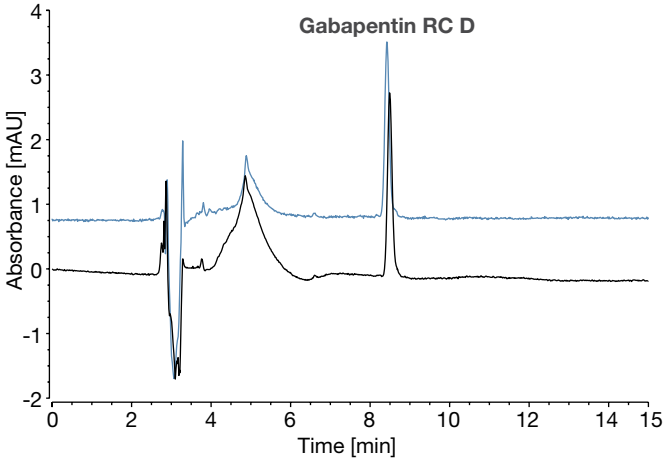

Figure 9. Injections of standard solution for the late eluting impurities on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system. The standard solution contains gabapentin related compound D.

Table 22. System suitability requirements for gabapentin analysis

Monograph requirement	Vanquish Core	UltiMate 3000
Assay: theoretical plates NLT 1900 for gabapentin, system suitability solution	6641	6650
Assay: area RSD NMT 0.73%, standard solution	0.02%	0.05%
Early eluting impurities: resolution (gabapentin related compound A – gabapentin related compound B) NLT 2.3, system suitability solution	4.09	3.76
Early eluting impurities: RSD NMT 5.0% for gabapentin related compound E with standard solution	3.94%	3.92%
Late eluting impurities: NLT 13600 theoretical plates with standard solution	24291	25039
RSD NMT 7.0% for standard solution	2.69%	0.76%

Conclusion

System suitability requirements were met with both instruments.

Table 23. Performance parameters for gabapentin analysis (n=6)

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Gabapentin, assay, standard solution	1.00	1.00	0.09	0.02	77.10	76.85	0.02	0.05
Gabapentin related compound A, early eluting impurities, system suitability solution	2.26	2.21	0.22	0.14*	0.125	0.128	2.78	2.23*
Gabapentin related compound B, early eluting impurities, system suitability solution	2.50	2.43	0.31	0.02*	0.094	0.088	2.80	1.19*
Gabapentin related compound E, early eluting impurities, standard solution	2.71	2.66	0.02	0.03	1.040	1.060	4.24	3.92
Gabapentin related compound D, late eluting impurities, standard solution	n.a.	n.a.	0.01**	0.04	0.414	0.380	0.99†	0.76

* indicates n=2
** indicates n=4
† indicates n=5

Furosemide (USP monograph[9])

Standards

- Furosemide, certified reference material, Sigma-Aldrich (P/N PHR1057-1G)
- Furosemide Related Compound A, certified reference material, Sigma-Aldrich (P/N PHR1817-100MG)
- Furosemide Related Compound B, certified reference material, Sigma-Aldrich (P/N PHR1816-100MG)

Assay

Sample preparation

Solution A was prepared by mixing ACN and ultrapure water 50/50 (v/v). The diluent was obtained by mixing solution A and glacial acetic acid (978:22). The standard solution was prepared by weighing 1.04 mg of USP Furosemide RS and diluting it in 1.04 mL diluent to obtain 1 mg/mL. 200 µL of this solution was added to 800 µL diluent to obtain the final concentration of 0.2 mg/mL.

The system suitability solution was prepared by mixing 20 µL of the 1 mg/mL USP Furosemide RS solution and 12 µL of a 1.03 mg/mL solution of Furosemide Related Compound A RS, and diluting with diluent to a final volume of 1.00 mL.

Chromatographic conditions

Table 24. Chromatographic conditions for furosemide assay and organic impurities

Parameter	Value
Column	Hypersil GOLD 250 × 4.6 mm, 5 µm (P/N 25005-254630)
Mobile phase	Water/THF/glacial acetic acid (70/30/1) (v/v/v)
Flow rate	1 mL/min
Column temperature	25°C
Autosampler temperature	10°C
Injection volume	20 µL
Detector settings	272 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Organic impurities

Sample preparation

Solution A, diluent, and system suitability solution were used the same as for the assay.

Chromatographic conditions

Chromatographic conditions are as given in Table 24 for the assay.

Results

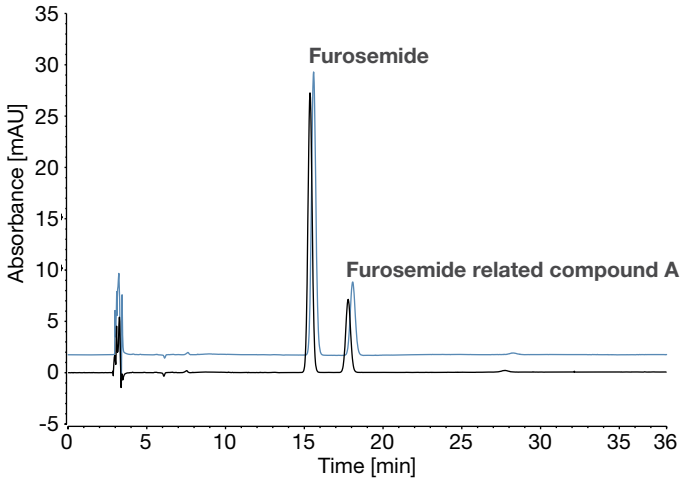

Figure 10. Injections of system suitability solution for furosemide assay on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 25. System suitability requirements for furosemide analysis (n=6)

Monograph requirement	Vanquish Core	UltiMate 3000
Assay: resolution (furosemide, furosemide related compound A) NLT 1.5, SST solution	4.46	4.48
Assay: area RSD NMT 0.73% with standard solution	0.07%	1.21%
Impurities: resolution (furosemide, furosemide related compound A) NLT 2.5, SST solution	4.46	4.48
Impurities: area RSD NMT 2.0% for furosemide with SST solution	0.07%	1.21%

Conclusion

System suitability requirements were met with the Vanquish Core HPLC system. System suitability requirements were met with the UltiMate 3000 HPLC system except for assay area precision.

Table 26. Performance parameters for furosemide analysis (n=6)

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Furosemide, assay SST	1.00	1.00	0.04	0.11	8.70	8.88	0.09	1.06
Furosemide, assay, standard solution	1.00	1.00	0.03	0.32	89.1	91.05	0.07	1.21
Furosemide related compound A, assay	1.16	1.16	0.04	0.13	2.61	2.66	0.16	1.10

Hydrochlorothiazide (EP method[10])

Standards

- Ph. Eur. Chlorothiazide CRS (P/N EDQM, C1700000)
- Ph. Eur. Hydrochlorothiazide CRS (P/N EDQM, H1200000)
- Ph. Eur. Hydrochlorothiazide for peak identification CRS (P/N EDQM, Y0001494)

Assay

Sample preparation

Reference stock solution A was prepared by accurately weighing 3.0 mg of chlorothiazide CRS and 3.0 mg hydrochlorothiazide CRS and dissolving in 2.5 mL of ACN and 2.5 mL of MeOH. After mixing, the solution was filled up to 20 mL with phosphate buffer solution pH 3.2. To obtain reference working solution A, 5.0 mL of the stock solution was diluted to 100 mL with solvent mixture, which was prepared by mixing 25 mL of MeOH, 25 mL of ACN with 200 mL phosphate buffer solution pH 3.2. Reference solution D was prepared by accurately weighing 3.0 mg of hydrochlorothiazide CRS in 250 µL of ACN and 250 µL MeOH. After mixing, the solution was filled up to 2.0 mL with phosphate buffer solution pH 3.2. The phosphate buffer was prepared by weighting 14.2 g of disodium hydrogen phosphate and dissolving in 900 mL ultrapure water. The pH was adjusted with 1:10 diluted phosphoric acid solution to pH 3.2 and afterwards the solution was filled to 1,000 mL with ultrapure water. Of this solution, 100 mL was diluted to 2,000 mL using ultrapure water.

Mobile phase preparation

To 940 mL of phosphate buffer solution pH 3.2, 60 mL of MeOH and 10 mL of THF were added and thoroughly mixed to obtain mobile phase A. Mobile phase B was prepared by mixing 500 mL of MeOH with 500 mL of phosphate buffer solution pH 3.2 and 50 mL of THF.

Chromatographic conditions

Table 27. Chromatographic conditions for hydrochlorothiazide assay

Parameter	Value
Column	Hypersil GOLD, 4.6 mm × 100 mm, 3 µm (P/N 25003-104630)
Mobile phase	A: Buffer/MeOH/THF (940/60/10) (v/v/v) B: Buffer/MeOH/THF (500/500/50) (v/v/v)
Gradient	Time (min) %A %B 0.0 80 20 4.0 80 20 10.0 20 80 10.1 80 20 18.0 80 20
Flow rate	1.6 mL/min
Column temperature	25°C
Autosampler temperature	10°C
Injection volume	10 µL
Detector settings	224 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Test for related substances

Mobile phases and samples are identical to assay.

Chromatographic conditions

Table 28. Chromatographic conditions for hydrochlorothiazide related substances

Parameter	Value
Column	Hypersil GOLD, 4.6 mm × 100 mm, 3 µm (P/N 25003-104630)
Mobile phase	A: Buffer/MeOH/THF (940/60/10) (v/v/v) B: Buffer/MeOH/THF (500/500/50) (v/v/v)
Gradient	Time (min) %A %B 0.0 100 0 17.0 55 45 30.0 55 45 30.1 100 0 45.0 100 0
Flow rate	0.8 mL/min
Column temperature	25°C
Autosampler temperature	10°C
Injection volume	10 µL
Detector settings	224 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Results

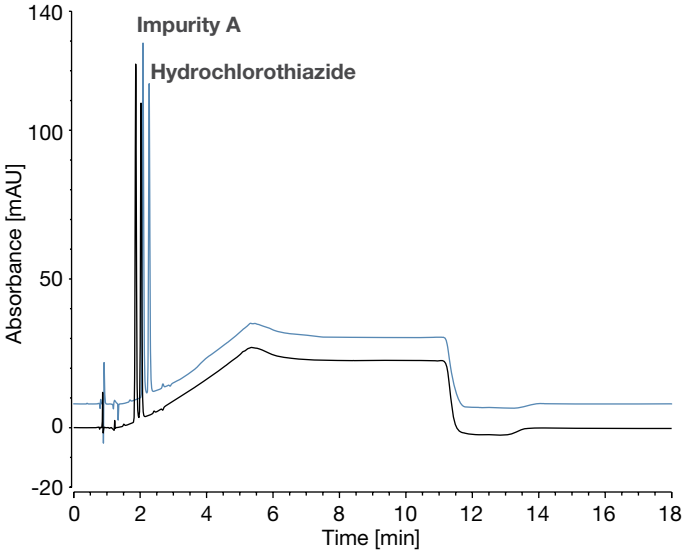

Figure 11. Injections of the reference working solution A for the assay of hydrochlorothiazide on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

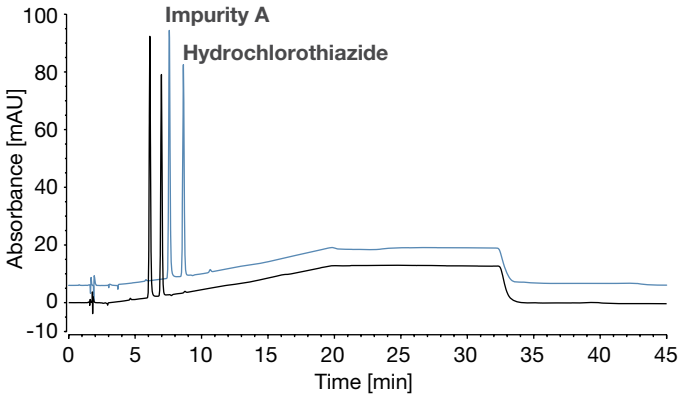

Figure 12. Injections of the reference working solution A for the related substances analysis of hydrochlorothiazide on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 29. System suitability requirements for hydrochlorothiazide analysis

Monograph requirement	Vanquish Core	UltiMate 3000
Related substances: resolution (impurity A, hydrochlorothiazide) NLT 2.5	4.78	5.46
Assay: resolution (impurity A, hydrochlorothiazide) NLT 2.0	2.38	2.64

Conclusion

System suitability requirements were met with both instruments.

Table 30. Performance parameters for hydrochlorothiazide analysis (n=6)

Peak	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Hydrochlorothiazide, assay	1.00	1.00	0.05	0.06	4.68	4.75	0.04	0.12
Hydrochlorothiazide, related substances	1.00	1.00	0.02	0.05	9.29	9.34	0.07	0.28
Impurity A, assay	0.92	0.92	0.04	0.04	5.30	5.45	0.04	0.15
Impurity A, related substances	0.88	0.88	0.02	0.05	10.64	10.89	0.06	0.27

Omeprazole (USP monograph[11])

Standards

- Omeprazole, certified reference material, Sigma-Aldrich (P/N PHR1059-1G)
- Omeprazole Related Compound A, certified reference material, Sigma-Aldrich (P/N PHR1648-30MG)
- Omeprazole Related Compound E, certified reference material, Sigma-Aldrich (P/N PHR1649-30MG)
- Omeprazole Related Compound I, certified reference material, Sigma-Aldrich (P/N PHR1650-30MG)

Assay

Sample preparation

The samples were prepared in a diluent composed of ACN and 0.01 M aqueous sodium borate in a ratio of 1:3 (v/v). A 500 mL volume of 0.01 M sodium borate was prepared by adding 400 mL ultrapure water to a 1,000 mL graduated cylinder containing a stir bar and 3.09 g boric acid. After stirring, 0.50 g sodium hydroxide was added. The solution was stirred again, and the volume was brought to 500 mL. The resulting pH was 8.1. A 100 mL volume of ACN was added to 300 mL of 0.01 M sodium borate in a 1,000 mL bottle. Diluent was added to the mark of a 25 mL volumetric flask containing 5.0 mg omeprazole to produce a 0.2 mg/mL sample of omeprazole. The 0.1 mg/mL system suitability solution was prepared by adding 500 µL of the 0.2 mg/mL sample to 500 µL of diluent in a sample vial.

Mobile phase preparation

A primary buffer solution was prepared by accurately weighing 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate and dissolving in 1,000 mL of ultrapure water. The pH was 7.6 and was not adjusted. An aliquot of 250 mL was taken and diluted with ultrapure water to 1,000 mL to obtain the buffer solution. The mobile phase was prepared by combining 750 mL of this buffer solution with 250 mL ACN.

Chromatographic conditions

Table 31. Chromatographic conditions for omeprazole assay

Parameter	Value
Column	Hypersil GOLD C8, 4.6 mm × 150 mm, 5 µm (P/N 25205-154630)
Mobile phase	Buffer/ACN (3/1) (v/v)
Flow rate	0.8 mL/min
Column temperature	25°C
Autosampler temperature	10°C
Injection volume	20 µL
Detector settings	280 nm, 4 nm bandwidth, 10 Hz, 0.5 s

Organic impurities

Mobile phase preparation

A 250 mL portion of the 4x buffer solution prepared for the assay was diluted to 1,000 mL and the pH adjusted to 7.0 using phosphoric acid to obtain mobile phase A. Mobile phase B was ACN.

Sample preparation

The diluent was prepared by combining 75 mL of mobile phase A and 25 mL ACN. The system suitability solution was prepared by adding 37.5 mg omeprazole to a 25 mL volumetric flask (1.5 mg/mL) and filling to the mark with diluent. Each impurity, E, I, and A, was prepared as a 0.6 mg/mL solution by adding 3.0 mg to a 5.0 mL volumetric flask and filling to the line with diluent. Finally, to a 25 mL volumetric flask was added 25 µL of each impurity and 10.0 mL of the omeprazole solution to yield 0.6 µg/mL for each impurity and 0.6 mg/mL for omeprazole after filling to the line with diluent. The standard solution, 0.6 µg/mL, was prepared in a 25 mL volumetric flask by adding 10 µL of the 1.5 mg/mL omeprazole stock solution and filling to the line with diluent. The sensitivity solution, 0.3 µg/mL, was prepared by adding 50 µL of the standard solution to 950 µL of diluent.

Chromatographic conditions

Table 32. Chromatographic conditions for omeprazole organic impurities

Parameter	Value		
Column	Hypersil GOLD C8, 4.6 mm × 250 mm, 5 µm (P/N 25205-254630)		
Mobile phase	A: Phosphate buffer B: ACN		
Gradient	Time (min)	%A	%B
	0.0	75	25
	12.0	75	25
	22.0	50	50
	45.0	50	50
	45.1	75	25
	55.0	75	25
Flow rate	0.8 mL/min		
Column temperature	25°C		
Autosampler temperature	4°C		
Injection volume	40 µL		
Detector settings	264 nm, 4 nm bandwidth, 10 Hz, 0.5 s		

Results

The signal to noise for the sensitivity solution of the impurities method was calculated with the peak-to-peak method over a fixed interval time range from 20 to 21 minutes. Omeprazole and related compound solutions were all used within 14 hours of preparation because they were unstable, even at 6°C.

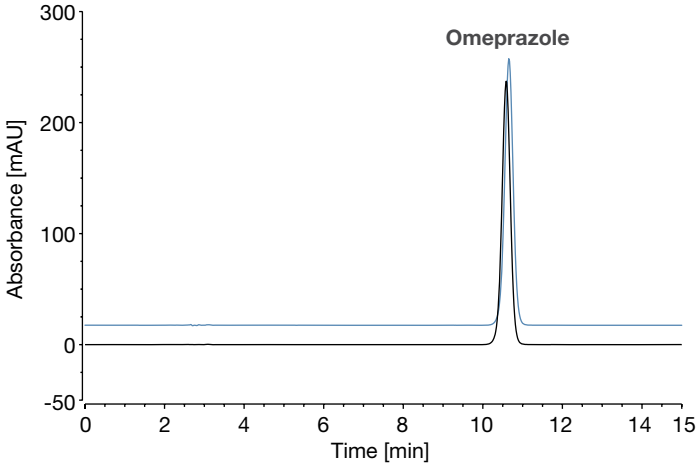

Figure 13. Injections of system suitability solution for the assay of omeprazole on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

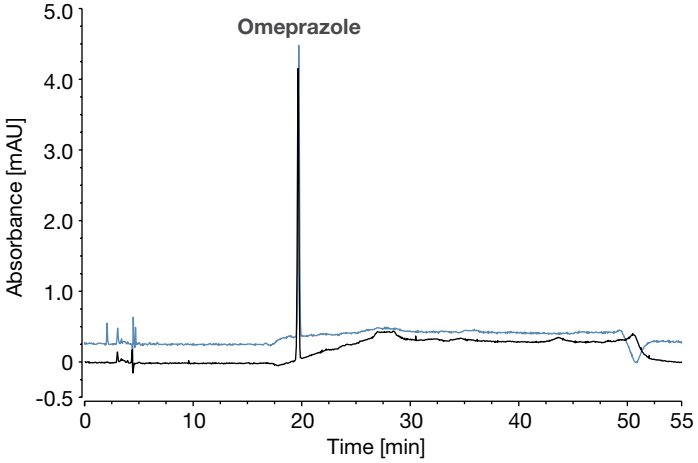

Figure 14. Injections of standard solution for the impurity method of omeprazole on a Vanquish Core (black) and an UltiMate 3000 (blue) HPLC system

Table 33. System suitability requirements for omeprazole analysis

Monograph requirement	Vanquish Core	UltiMate 3000
Assay: tailing factor for omeprazole NMT 1.5, SST solution	0.98	0.97
Assay: area RSD for omeprazole NMT 1.0%, SST solution (n=6)	0.02%	0.06%
Impurities: resolution (omeprazole – omeprazole related compound A) NLT 2.0, SST solution	2.65	2.25
Impurities: area RSD for omeprazole NMT 5.0%, standard solution (n=6)	0.27%	3.66%
Impurities: signal to noise for omeprazole NLT 10, sensitivity solution	13.3	16.6

Table 34. Performance parameters for omeprazole analysis (n=6)

	RRT		RT RSD (%)		Area (mAU·min)		Area RSD (%)	
Peak	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000	Vanquish Core	UltiMate 3000
Omeprazole, assay, SST solution	1.00	1.00	0.00	0.04	59.73	60.96	0.02	0.06
Omeprazole, impurities, standard solution	1.00	1.00	0.01	0.03	0.66	0.63	0.27	3.66

Conclusions

- EP and USP assay and impurities HPLC methods were run for nine of the most prescribed drugs worldwide.
- All compendial methods could be successfully implemented with Thermo Scientific HPLC columns.
- All system suitability criteria were easily met for all drugs with the Vanquish Core HPLC system.
- All system suitability criteria were easily met for all drugs with the UltiMate 3000 SD HPLC system. The only exception was furosemide assay area precision on the UltiMate 3000 system.
- All methods described in this work can be confidently implemented with Thermo Scientific HPLC systems and columns.
- UltiMate 3000 HPLC system users can easily transfer compendia methods to Vanquish Core HPLC systems.

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