Application Note

Biopharma/Pharma

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Performance Evaluation of the Agilent InfinityLab Pro iQ Series

Assessment of key performance metrics in the simultaneous separation of eight drug substances

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Abstract

This application note demonstrates the performance of the Agilent InfinityLab Pro iQ Series for routine small-molecule pharmaceutical analysis. Using a robust reversed-phase HPLC method coupled with a single quadrupole mass spectrometer, eight commonly used drug substances were simultaneously separated and quantified within nine minutes. The system exhibited excellent sensitivity, achieving limits of quantification as low as 0.02 ng/mL, strong linearity across wide concentration ranges, and reproducibility with RSD values below 3%. Comparative evaluation of electrospray ionization (ESI) and Agilent Jet Stream (AJS) sources revealed a 2- to 5-times signal enhancement with AJS, confirming the system's suitability for walk-up analytical laboratories, medicinal chemistry workflows, and QA/QC environments worldwide.

Introduction

Pharmaceutical laboratories increasingly require fast, reliable, and sensitive analytical solutions to support diverse workflows, from early-stage medicinal chemistry to quality assurance and manufacturing control. Small-molecule drugs, which dominate therapeutic portfolios globally, demand robust methods capable of handling complex matrices, low-level detection, and high-throughput operation. Traditional LC/MS approaches often involve trade-offs between sensitivity, ease of use, and cost, creating a need for systems that combine performance with simplicity for routine applications.

The Agilent InfinityLab Pro iQ Series addresses these challenges by integrating advanced chromatographic capabilities with a high-sensitivity single quadrupole mass spectrometer. This study evaluates its performance through simultaneous analysis of eight pharmaceutically active ingredients representing a range of chemical properties and ionization behaviors. Key metrics such as sensitivity, linearity, and reproducibility were assessed under optimized conditions, and the impact of ionization source selection (ESI versus AJS) was explored to highlight the system's versatility for general-purpose and front-line analysis.

Experimental

Reagents and standards

Ammonium fluoride, ammonium formate, and formic acid were obtained from Sigma-Aldrich. Methanol was supplied by Agilent, and ultrapure water was produced using an in-house Milli-Q system and used for the analysis. Amoxicillin, aspirin, buspirone, captopril, chloramphenicol, erythromycin, ibuprofen, and omeprazole were sourced from various vendors. Figure 1 shows the chemical structure of these analyzed active pharmaceutic ingredients, while Table 1 contains some further information on the compounds (CAS, molecular formula, detected ion species).

Table 1. Additional information for the analyzed drug substances.

Compound Name	CAS No.	Molecular Formula	Detected *m/z*	Corresponding Species
Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	366.1118 731.2164	$[M+H]^+$ $[2M+H]^+$
Aspirin	50-78-2	$C_9H_8O_4$	121.0284	$[M-COOCH_3]^+$
Buspirone HCl	33386-08-2	$C_{21}H_{32}ClN_5O_2$	386.2556	$[M-Cl]^+$
Captopril	62571-86-2	$C_9H_{15}NO_3S$	218.0845	$[M+H]^+$
Chloramphenicol	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$	321.0051	$[M-H]^-$
Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	734.4685	$[M+H]^+$
Omeprazole	73590-58-6	$C_{17}H_{19}N_3O_3S$	346.122	$[M+H]^+$
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	205.1234	$[M-H]^-$

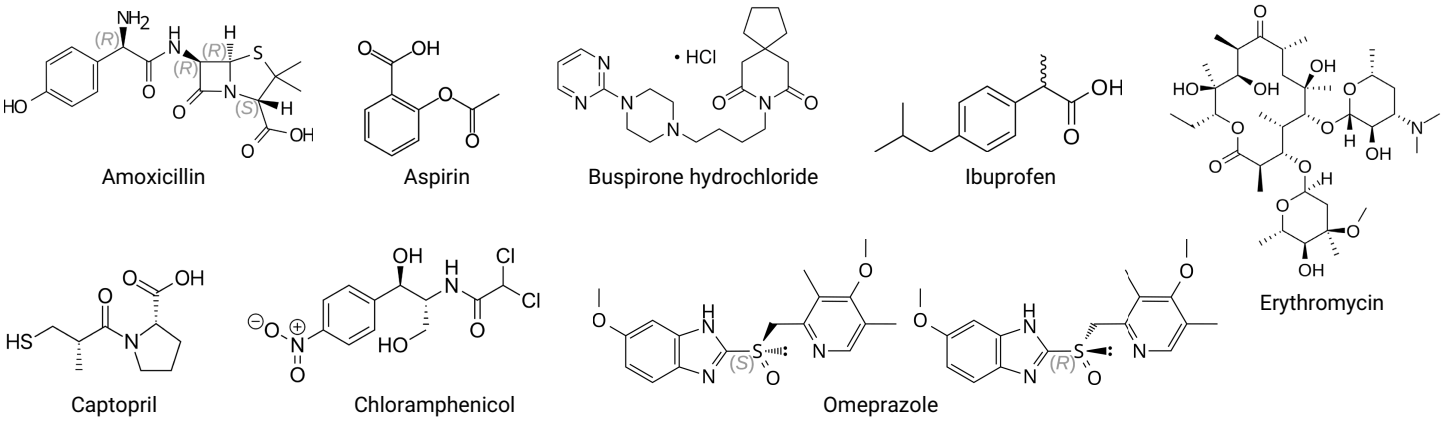

Figure 1. Chemical structures of amoxicillin, acetylsalicylic acid (aspirin), buspirone, captopril, chloramphenicol, erythromycin, ibuprofen, and omeprazole.

A concentrated stock solution (1 mg/mL) was prepared for each standard using methanol as the solvent, except for amoxicillin and omeprazole, which required dimethyl sulfoxide due to solubility limitations. Subsequently, a combined stock solution at 10 µg/mL was prepared in a 1:1 mixture of water and methanol to include all standards. Further dilutions of this mixed standard were made using a 9:1 water:methanol solution, covering a concentration range from 0.02 to 1,000 ng/mL. Table 2 summarizes the exact preparation and dilution series.

Table 2. Summary of the preparation of the dilution series.

Calibration Standard Name	STD Concentration (ng/mL)	STD Taken for Dilution	Volume STD (µL)	Volume Solvent (Water:Methanol 9:1)
API-STD-1000	1000	Stock solution	100	900
API-STD-500	500	Stock solution	50	950
API-STD-200	200	Stock solution	20	980
API-STD-100	100	API-STD-1000	100	900
API-STD-50	50	API-STD-1000	50	950
API-STD-20	20	API-STD-1000	20	980
API-STD-10	10	API-STD-100	100	900
API-STD-5	5	API-STD-100	50	950
API-STD-2	2	API-STD-100	20	980
API-STD-1	1	API-STD-10	100	900
API-STD-0.5	0.5	API-STD-10	50	950
API-STD-0.2	0.2	API-STD-10	20	980
API-STD-0.1	0.1	API-STD-1	100	900
API-STD-0.05	0.05	API-STD-1	50	950
API-STD-0.02	0.02	API-STD-1	20	980

Equipment and method

An Agilent 1290 Infinity II LC system equipped with Agilent OpenLab CDS software (version OpenLab CDS 2.8 Update08 FP2) was used for the analysis. The instrumentation details are provided in Table 3.

Table 3. Instrument configuration details.

Module	Product Number
Agilent 1290 Infinity II high-speed pump	G7120A
Agilent 1290 Infinity II vialsampler with InfinityLab integrated column compartment (with 3 µL heat exchanger)	G7129B
Agilent 1290 Infinity II DAD with InfinityLab Max-light cartridge cell, 10 mm	G7117B
Agilent InfinityLab Pro iQ Plus with ESI and Agilent Jet Stream source	G6170A

An Agilent ZORBAX Eclipse Plus C18 RRHD column (2.1 × 100 mm, 1.8 µm; p/n 959758-902) was employed for the evaluation.

The source and acquisition parameters for the Pro iQ Plus system are listed in Tables 4 and 5, and the high-performance liquid chromatography (HPLC) parameters are listed in Table 6.

Table 4. MS source parameters for the Agilent InfinityLab Pro iQ Plus used in this study.

Parameter	Value
MS	InfinityLab Pro iQ Plus (G6170A)
Source	ESI/Agilent Jet Stream
Drying Gas Flow	11.0 L/min
Gas Temperature	250 °C
Nebulizer Pressure	45 psi
Capillary Voltage	4,500 V
Sheath Gas Temperature	400 °C
Sheath Gas Flow	12 L/min
Nozzle Voltage	0 V
Mode	Positive/negative
Scan	*m/z* 100–800
Scan Time	225 ms
Fragmentor	70/90/110/150 V
Gain Factor	1

Table 5. Acquisition parameters in case of selected ion monitoring (SIM) used in this study.

Compound	Polarity	Mass (*m/z*)	Fragmentor (V)	Quadrupole Resolution	Dwell Time (ms)
Amoxicillin 366	Positive	366.1	90	Unit	21
Amoxicillin 731	Positive	731.2	90	Unit	
Aspirin	Positive	121	110	Unit	
Buspirone	Positive	386.3	150	Unit	
Captopril	Positive	218	90	Unit	
Chloramphenicol	Negative	321	90	Unit	
Erythromycin	Positive	734.5	130	Unit	
Ibuprofen	Negative	205.1	70	Unit	
Omeprazole	Positive	346.1	90	Unit	

Table 6. HPLC parameters used in this study.

Parameter	Value
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Sampler Temperature	4 °C
Mobile Phase 1	A) 5 mM ammonium formate in water + 0.1% formic acid B) 5 mM ammonium formate in methanol + 0.1% formic acid
Mobile Phase 2	A) Water + 0.1% formic acid B) Methanol + 0.1% formic acid
Mobile Phase 3	A) 0.5 mM ammonium fluoride in water B) 0.5 mM ammonium fluoride in methanol
Flow Rate	0.6 mL/min
Injection Volume	10 µL
Needle Wash	Standard wash, 3 sec
Column Temperature	40 °C
UV Detection	254 nm
Post Time	1 min
Gradient Program	Time (min) %B 0 5 0.5 5 1 30 6 60 8 95 8.5 95 8.6 5

Three different mobile phase additives were tested to account for the varying properties of the analyzed compounds. For captopril, 5 mM ammonium formate with 0.1% formic acid gave the best performance. For erythromycin, the optimal additive was 0.1% formic acid. For the remaining compounds (amoxicillin, aspirin, buspirone, chloramphenicol, ibuprofen, and omeprazole), 0.5 mM ammonium fluoride provided the best results. Negative ionization mode was used for chloramphenicol and ibuprofen, while positive ionization mode was applied for the rest of the compounds.

Summary of results

A short screening method was used to acquire UV and MS scan data of the mixture of drug substances at the concentration level of 10 µg/mL. Figure 2 shows the results, where two small-molecule pharmaceuticals (captopril and erythromycin) are not UV active and can only be detected by MS.

To identify the most intense MS signal for each compound, the mass spectrum was extracted from the total ion chromatogram (TIC) for each peak. A summary of the mass spectra is presented in Figure 3.

The compounds were analyzed over concentration ranges from 0.02 to 500 ng/mL, depending on the analyte and linearity, sensitivity, and reproducibility were evaluated. Figure 4 shows the comparison of the Selected Ion Monitoring (SIM) signals at the concentration level of 10 ng/mL, using the most optimal mobile phases as well as optimum MS parameters for each analyte.

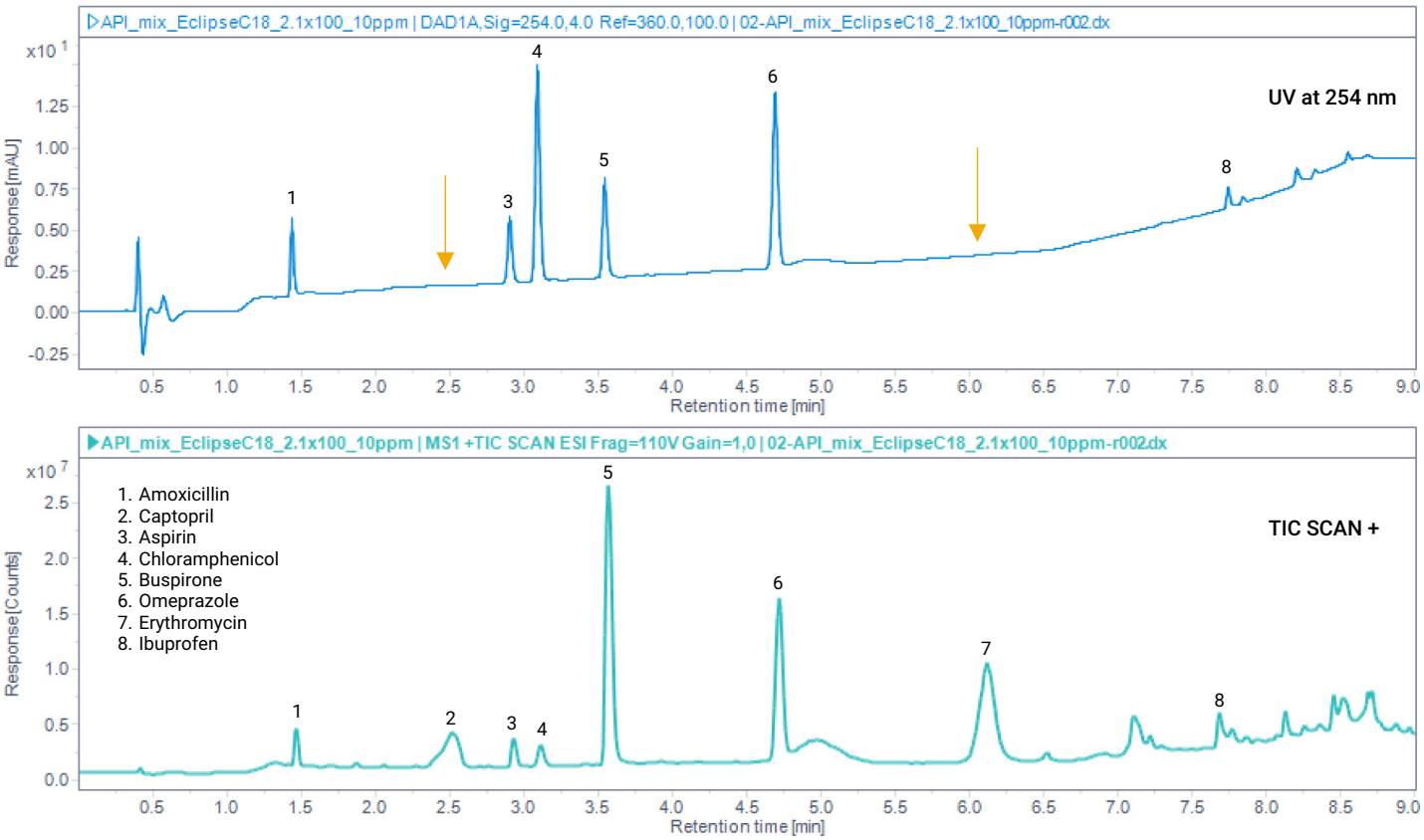

Figure 2. Chromatographic separation of the eight small-molecule drug substances.

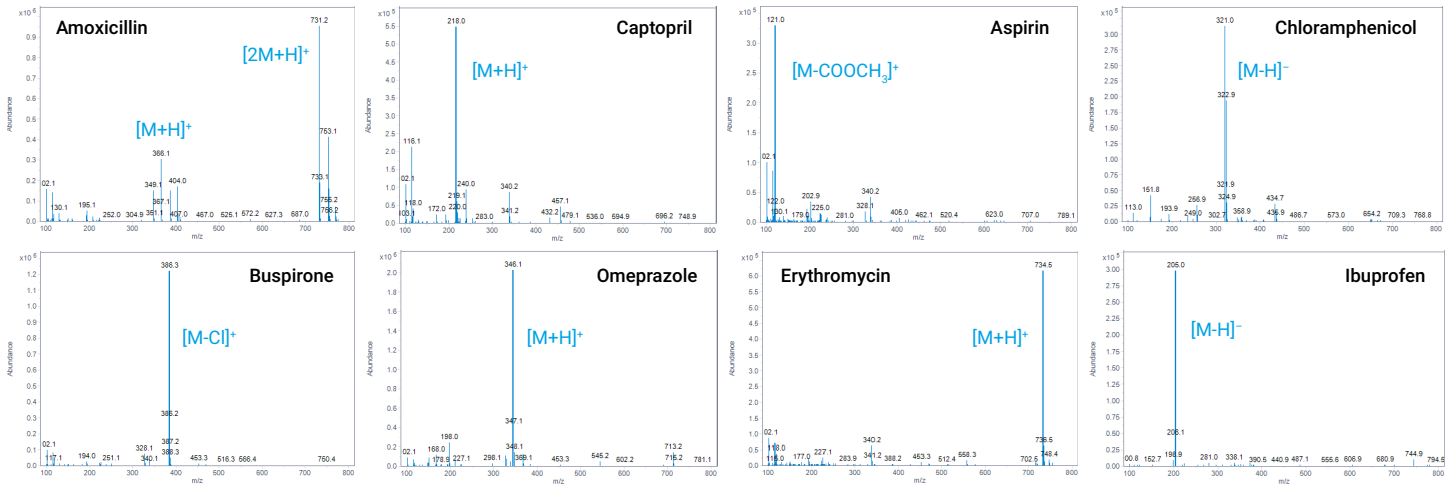

Figure 3. Mass spectra of the eight small-molecule drug substances.

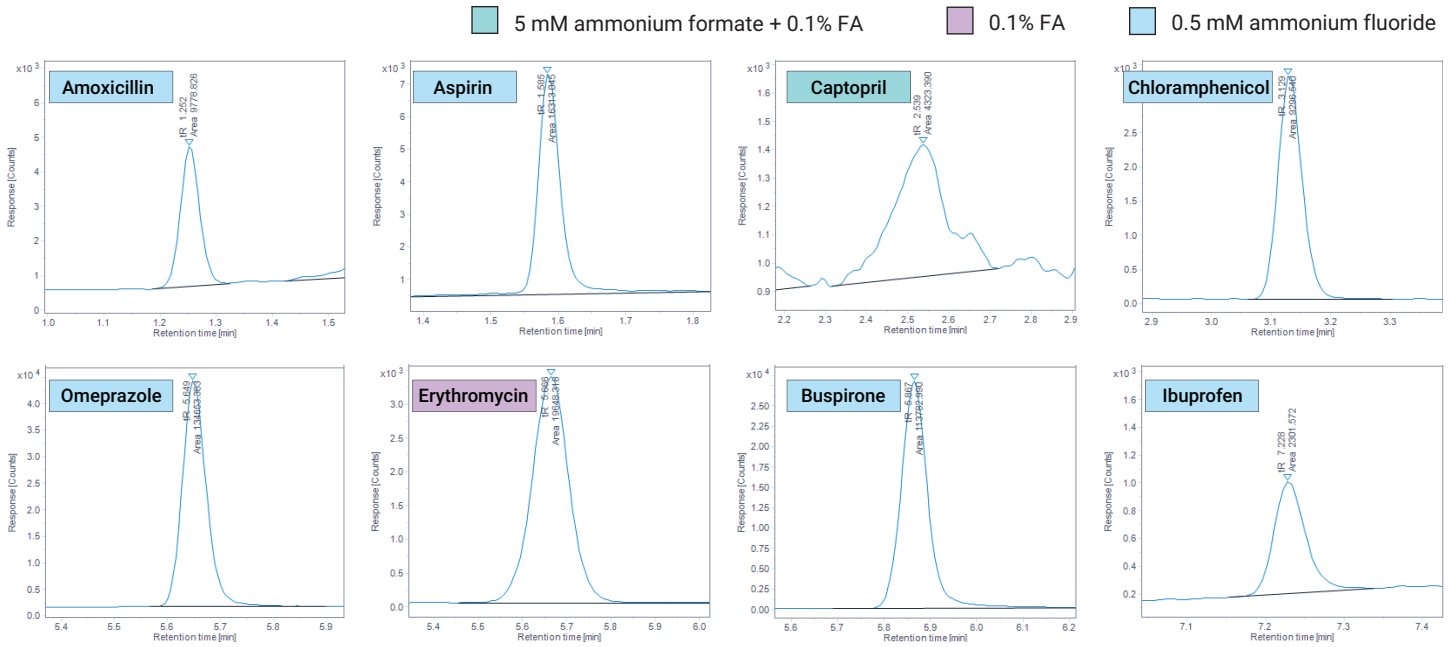

Figure 4. Comparison of the analyzed compounds at the concentration level of 10 ng/mL.

Linearity and sensitivity

Figure 5 shows the linearity curves within the concentration range of 0.02 to 500 ng/mL or 0.02 to 200 ng/mL, all exhibiting an R^2 value greater than 0.995. A linear response was observed for all compounds except aspirin, for which a quadratic model provided the best fit. To achieve a linear range for aspirin, it would likely be necessary to reduce the concentration range even more.

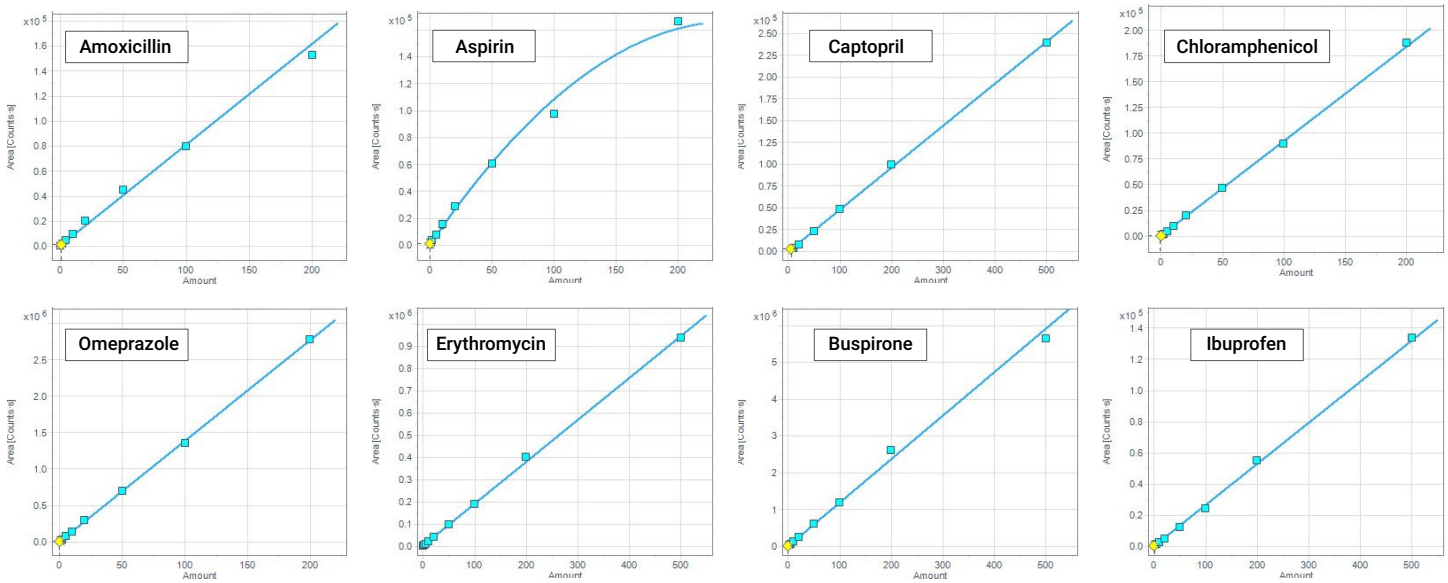

Figure 5. Calibration curves for all compounds.

Table 7 summarizes the linearity data and the limit of quantification (LOQ), along with the corresponding S/N value for each compound at this level. The noise range was evaluated between 4.0 and 4.3 minutes using the default P2P algorithm while LOQ was defined as the lowest concentration yielding an S/N ≥ 9.9. Buspirone exhibited the widest linear dynamic range, spanning more than four orders of magnitude. Figure 6 presents representative chromatograms for each compound at their respective LOQ levels.

Table 7. Summary table of linearity and sensitivity.

Compound Name	Linear Range (ng/mL)	R^2	LOQ (ng/mL)	S/N at LOQ
Amoxicillin	1–200	0.995	1	16.6
Aspirin	0.5–200	0.995	0.5	11.4
Captopril	5–500	0.999	5	9.9
Chloramphenicol	0.1–200	0.999	0.1	11.5
Omeprazole	0.1–200	0.999	0.1	11.4
Erythromycin	0.1–500	0.999	0.1	12.6
Buspirone	0.02–500	0.996	0.02	40.2
Ibuprofen	1–500	0.999	1	15.6

Up to this point, the results have been presented using the ESI. Figure 7 compares these results (blue) with those obtained using the AJS source (purple) at a concentration level of 10 ng/mL. We can conclude that employing the AJS source provides an additional two- to five-fold increase in signal intensity for the analyzed pharmaceutical compounds.

Reproducibility

Reproducibility was evaluated using four replicate injections at concentration levels of 0.5 and 1 ng/mL for six pharmaceutical compounds, where 0.5 mM ammonium fluoride appeared to be the optimal mobile phase additive. The results demonstrated excellent RSD (%) values, consistently at or below 3%. (Table 8)

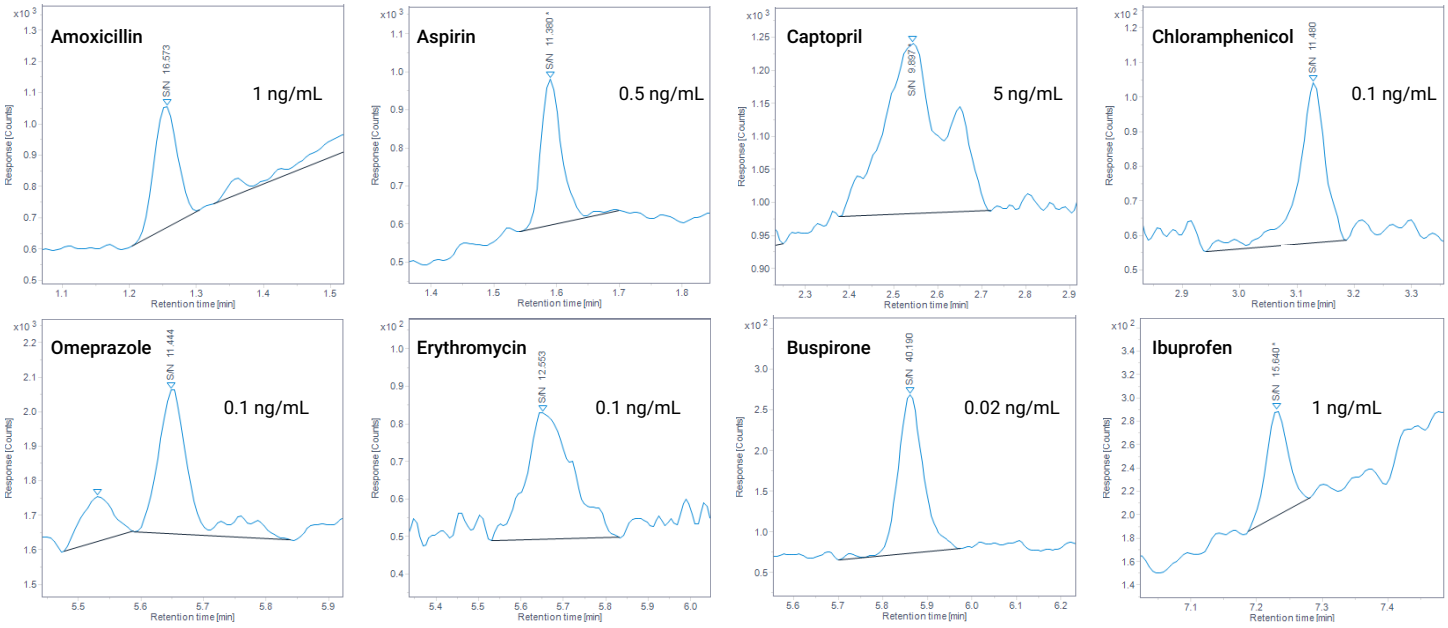

Figure 6. Representative chromatograms at LOQ levels using electrospray ionization.

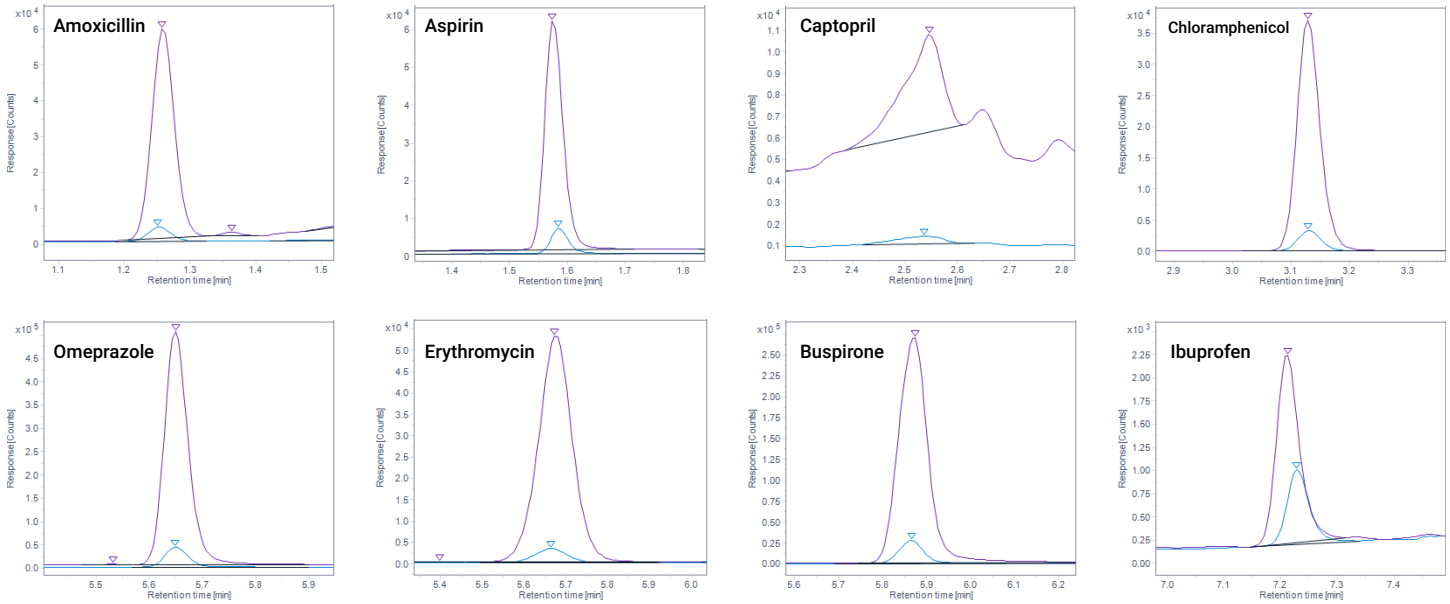

Figure 7. Comparison of the pharmaceuticals at 10 ng/mL using ESI (blue) versus Agilent Jet Stream (purple).

Table 8. Summary table of reproducibility.

	RSD (%) (n = 4)	
Compound Name	**0.5 ng/mL**	**1 ng/mL**
Amoxicillin	2.6	1.3
Chloramphenicol	0.8	0.6
Omeprazole	0.8	0.2
Aspirin	1.3	0.7
Buspirone	0.7	0.3
Ibuprofen	3.2	3.0

Conclusion

The Agilent InfinityLab Pro iQ Series delivers exceptional sensitivity, robustness, and ease of use for small-molecule pharmaceutical applications. The series' ability to achieve low-level quantification, maintain linearity across broad concentration ranges, and provide reproducible results positions it as an ideal solution for walk-up analytical environments, medicinal chemistry labs, and QA/QC workflows. Enhanced performance with the Agilent Jet Stream source further underscores its suitability for demanding applications such as cleaning validation and trace-level impurity monitoring, ensuring confidence in compliance and product integrity.

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