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LC-MS/MS determination of Synercid® injections

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Abstract

Synercid® is a combination of two semisynthetic pristinamycin derivatives, quinupristin and dalfopristin in 30:70 (w/w) ratio. A rapid and specific high-performance liquid chromatography-mass spectrometry was developed for the determination of quinupristin and dalfopristin using positive electrospray tandem mass spectrometry (+ESI-MS/MS). Multiple reaction monitoring transitions at $1023.05 > 134.34$ and $691.87 > 166.26$ were selected for the quantitation of quinupristin and dalfopristin, respectively. The assay run cycle-time was ~2.0 min injection-to-injection. The assay was linear up to concentration of 4000 ng ml^{-1} quinupristin and 1920 ng ml^{-1} dalfopristin. The lowest limits of quantitation of quinupristin and dalfopristin were found to be 1000 and 480 ng ml^{-1}, respectively. Quantitation was based on peak area measurement of quinupristin and dalfopristin using weighed linear regression. Linear relationships with correlation coefficients ($r > 0.99$) were automatically computed for both constituents by MASSLYNX quantify program. The ratio of the slopes of the calibration curves of quinupristin and dalfopristin was found to be 0.425, which matches the nominal ratio composition of the antimicrobial compounds in Synercid®. The %RSD ranges were 2.3–4.0% for dalfopristin and 1.3–4.2% for quinupristin, whereas the %DEV ranges were (−7.5+3.7) and (−1.2+9.1%), respectively, indicating appropriate precision and accuracy. Recoveries of 99.5–103.8% and 97.8–99.0% of quinupristin and dalfopristin, respectively, were computed from Synercid® injection. The described method is recommended for rapid determination of the contents and for tracking the stability and compatibility of quinupristin and dalfopristin in Synercid® injection.

Keywords: Synercid®; Quinupristin; Dalfopristin; LC-MS/MS

1. Introduction

Synercid® (formerly RP59000; Rhone-Poulenc) is the first semisynthetic injectable streptogramin antimicrobial agent with excellent activity against gram-positive bacteria [1,2]. Synercid® is a combination of two semisynthetic derivatives of pristinamycin, quinupristin and dalfopristin, in a fixed 30:70 (w/w) ratio [3] (Fig. 1). The active constituents of Synercid® have only limited antibacterial activity when administered individually, but act synergistically when combined to provide bactericidal activity. It acts at different sites on bacterial

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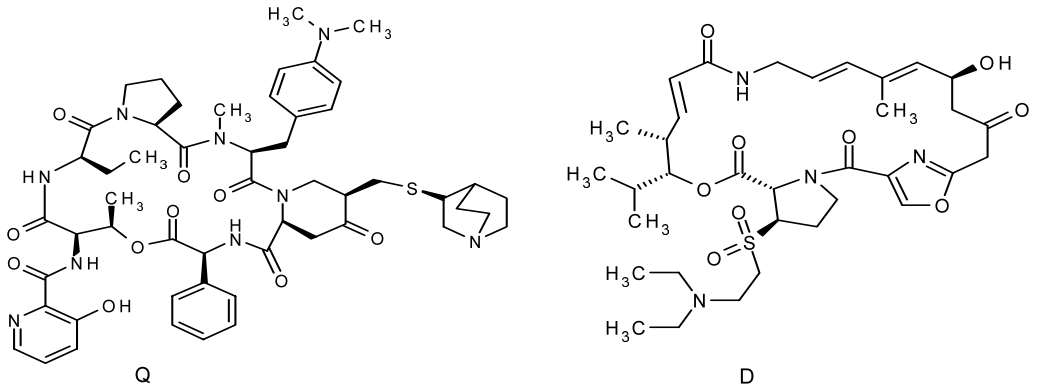

Fig. 1. Structures of quinupristin (Q) and dalfopristin (D).

ribosomes, thus inhibiting protein synthesis. Synercid® is active against a wide range of sensitive- and resistant-gram-positive cocci including methicillin-resistant staphylococci and penicillin-sensitive, -intermediate, -resistant *Streptococcus pneumoniae* [2]. However, it is not active against gram-negative bacilli. The activity of Synercid® against enterococci including *Enterococcus faecalis*, *Enterococcus avium* and vancomycin-resistant *Enterococcus faecium* has been also reported [2,4]. For pharmaceutical and clinical studies, accurate and specific methods are required for monitoring quinupristin and dalfopristin in the samples. There is paucity of literature reports on the analysis of quinupristin and dalfopristin. This might be attributed to the molecular complexity (FW: 1022, 690), low chemical reactivity and poor stability of compounds. An HPLC assay was developed to establish the pharmacokinetics of quinupristin and dalfopristin in biological samples [3]. In this assay, fluorescence and UV detectors were used. In another report, a gradient HPLC procedure was applied in routine quality control (QC) of freeze-dried powder of Synercid® under clinical conditions of administration [4]. In this analysis, quinupristin and dalfopristin were detected at two different wavelengths using photodiode-array detector. The major components, quinupristin and dalfopristin, were eluted at relatively long retention times and were quantified at concentration levels of 5–15 μg ml^{-1}. Recently, mass spectrometry has been used in combination with liquid chromatography for qualitative and quantitative drug analysis. In our laboratories, ion-trap mass spectrometry was used to determine the concentrations of furosemide [5], celecoxib [6] and linezolid [7] in biological samples. Furthermore, the technique was applied to investigate the chemical structure and stability of selected enaminones [8], 4-pyranones [9] and 2,6-piperidindiones [10] derivatives. The technique proved to be more advantageous for qualitative rather than quantitative analysis. Electrospray tandem mass spectrometry (ESI-MS/MS) using triple quadrupole mass spectrometer was recognized as a powerful tool for quantitative pharmaceutical and biopharmaceutical analysis of medicinal compounds In this work, we report on the application of positive electrospray tandem mass spectrometry in the analysis of quinupristin and dalfopristin; and application of liquid chromatography-multiple reaction monitoring (HPLC-MRM) in quantification, stability and compatibility studies of quinupristin and dalfopristin in freeze-dried powder of Synercid®.

2. Experimental

2.1. Chemicals and reagents

Synercid® (500 mg) was provided by Aventis Pharma GmbH (Germany) as freeze-dried powder for injection. The powder was stored in refrigerator at −20 °C. HPLC grade acetonitrile (Fisher

Scientific) was used for preparation of the mobile phase. Purified water obtained from Millipore system was used throughout the experiment.

2.2. Equipment

A triple quadrupole tandem mass spectrometer (Quattro LC, Micromass, UK) fitted with a Z-spray ion source was used. The mass spectrometer was operated in positive electrospray ionization mode (+ESI) and was coupled to Waters 2690 Separation Module, Alliance HPLC and Waters autosampler. System operation and data acquisition were controlled by MASSLYNX NT 3.5 software. The tuning parameters for MS/MS and multiple reaction monitoring (MRM) analyses of quinupristin and dalfopristin were optimized by direct infusion of a solution of Synercid® in the mobile phase to the ionization probe using Harvard syringe pump at flow rate 20 μl min^{-1}. Chromatographic analyses were performed using XTerra™ MS, C18, 2.5 μm (2.1 × 30 mm) column (Waters) and a mobile phase of acetonitrile/water (70:30 v/v) at flow rate 0.4 ml min^{-1} at ambient temperature.

2.3. Preparation of standards

A stock solution of Synercid® was prepared by dissolving ~10 mg of the powder in the mobile phase to give a concentration of 1 μg μl^{-1}. A 50 μl aliquot was diluted to 1 ml with mobile phase to give the working solution of 50 ng μl^{-1}. The stock and working solutions should be freshly prepared prior to the analysis.

2.4. Calibration curves

Aliquots of the working solution were appropriately diluted with mobile phase to obtain the concentrations of 1000–4000 ng ml^{-1} of quinupristin and 480–1920 ng ml^{-1} of dalfopristin. The calculated concentrations of quinupristin and dalfopristin in the calibration curves were based on quinupristin/dalfopristin ratio in Synercid®. Aliquots of 10-μl volumes were automatically injected and analyzed at MRM transitions, 1023.05 > 134.34 and 691.87 > 166.26, for quinupristin and dalfopristin, respectively. Calibration curves were automatically constructed by the MASSLYNX quantify program using weighted regression mode. Only calibration curves with correlation coefficients ($r > 0.99$) were considered.

2.5. Precision and accuracy studies

Control samples ($n = 7$) of Synercid® were prepared in the mobile phase at concentrations of 1000–2000 ng ml^{-1} (dalfopristin) and 1200–1700 ng ml^{-1} (quinupristin) and were analyzed, as mentioned above. The measured concentrations were used to calculate %RSD and %DEV values for each component to evaluate the precision and accuracy of LC-MS/MS method.

2.6. Stability studies

Two sets of samples at concentrations 2000 ng ml^{-1} (dalfopristin) and 4000 ng ml^{-1} (quinupristin) were prepared in (acetonitrile/water, 70:30 w/w) and were kept at 25, 4 and −20 °C for 10 days. Samples were withdrawn and analyzed using the developed LC-MS/MS. The peak areas of quinupristin or dalfopristin were automatically measured by MASSLYNX software.

2.7. Quality control samples

An aqueous sample of Synercid® was prepared by dissolving ~100 mg of the powder in 10 ml of the mobile phase. Synercid® samples containing quinupristin and dalfopristin at concentrations 1200, 2000, 4000 ng ml^{-1} of quinupristin and 2000, 4000 ng ml^{-1} of dalfopristin, were prepared and analyzed by LC-MS/MS using the specific MRM transitions. The concentrations of dalfopristin and quinupristin were automatically calculated from the regression equations of the calibration curves using MASSLYNX quantify program. Recoveries were calculated by reference to the nominal concentrations.

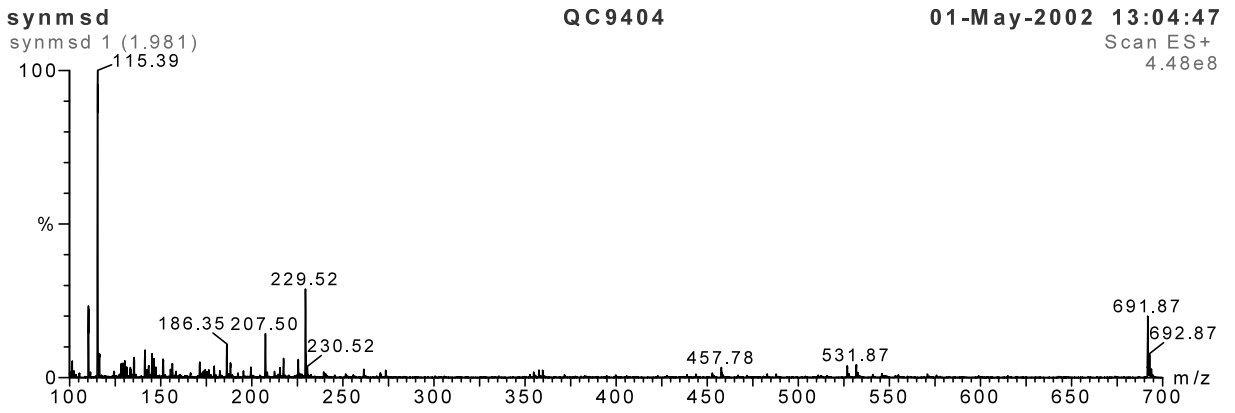

Fig. 2. MS scan of dalfopristin using +ESI.

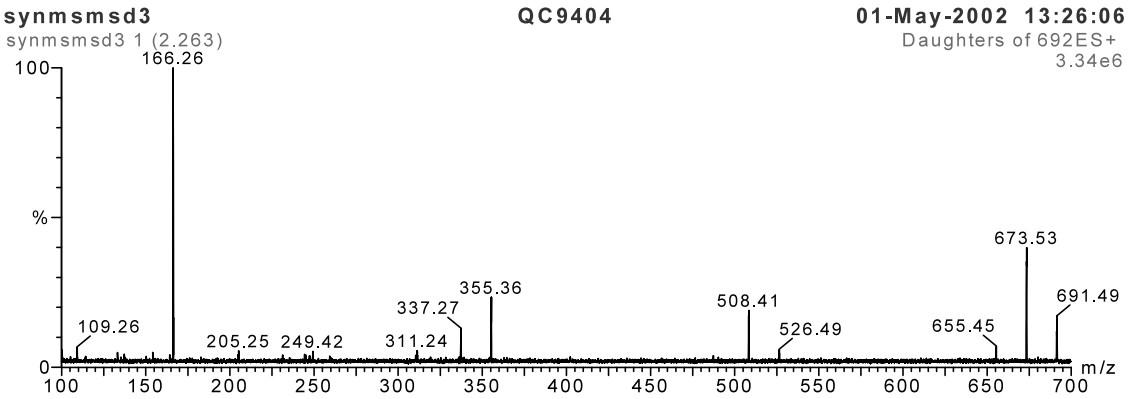

Fig. 3. MS/MS scan of dalfopristin using +ESI (parent ion *m*/*z* 691).

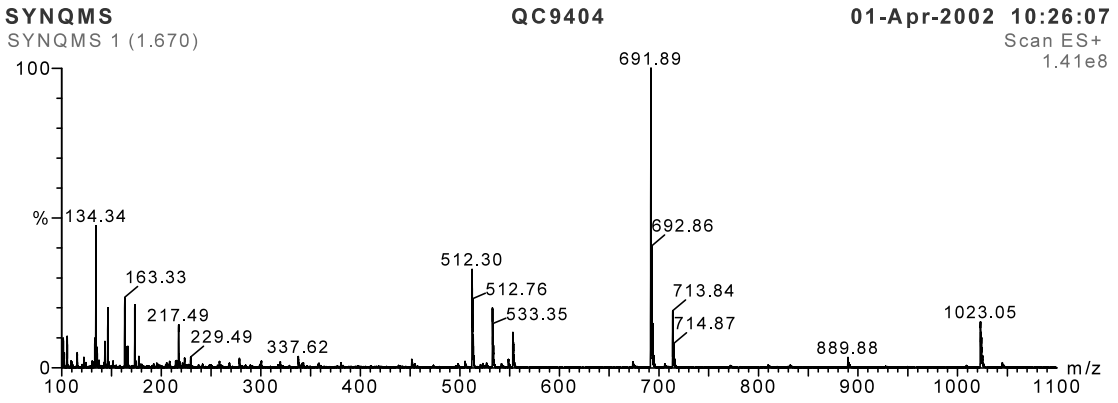

Fig. 4. MS scan of quinupristin using +ESI.

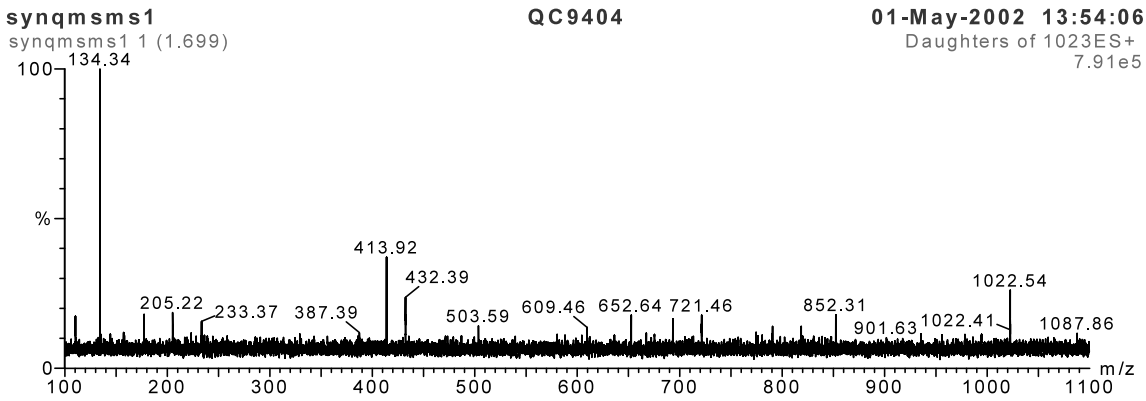

Fig. 5. MS/MS scan of quinupristin using +ESI (parent ion *m*/*z* 1023).

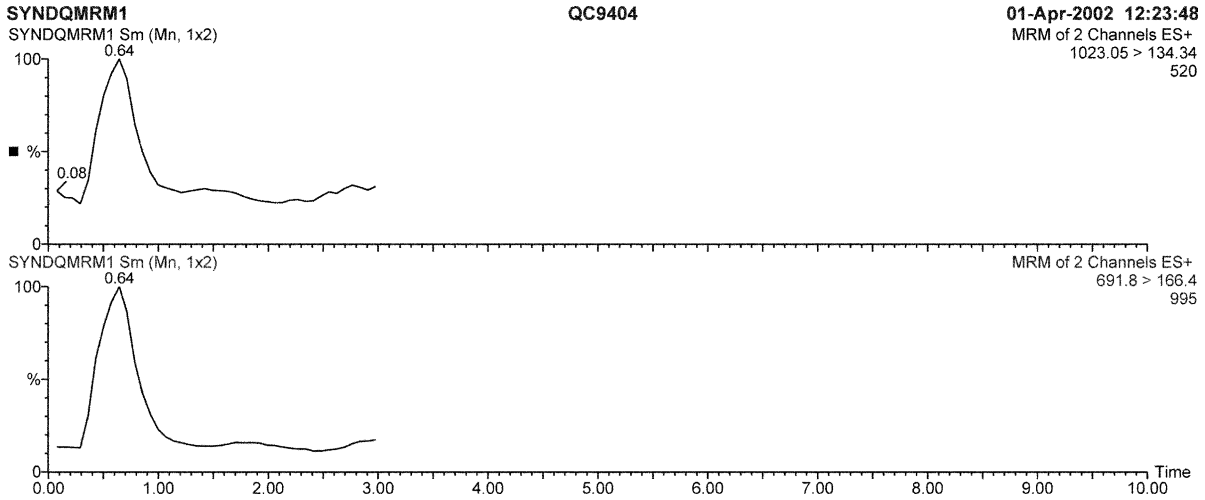

Fig. 6. HPLC-MRM of quinupristin and dalfopristin in Synercid®.

3. Results and discussion

3.1. Development of LC-MS/MS

The tuning parameters for the electrospray ionization of dalfopristin and quinupristin in positive mode (+ESI) were optimized for maximum detection of the parent and daughter ions. The tuning parameters; capillary voltage, cone voltage and collision energy were optimized at 3.1 kV, 32 V and 25 eV for dalfopristin and 3.2 kV, 32 V and 45 eV for quinupristin. The source and desolvation temperatures were adjusted at 100 and 250 °C, respectively. Using the above tuning parameters, the parent ions of dalfopristin and quinupristin were detected at *m*/*z* 691.87 and 1023.05, respectively (Fig. 2Fig. 4). These molecular mass ions were further used in -MS/MS experiments to determine the daughter ions of dalfopristin and quinupristin, which were detected at *m*/*z* 166.26 and 134.34, respectively (Figs. 3 and 5). MRM scan was then selected to determine specifically dalfopristin or quinupristin in Synercid® powder (Fig. 6). As MRM relates daughter ion to the target compound, therefore it is possible to determine dalfopristin and quinupristin independently in mixtures. MRM transitions at 691.87 > 166.26 and 1023.05 > 134.34 were selected to quantify dalfopristin and quinupristin with high degree of selectivity. HPLC parameters such as

Table 1
Linearity parameters for determination of quinupristin and dalfopristin by LC-MS/MS method ($n = 8$)

Compound	Concentration range	Slope	Correlation coefficient (r)
Quinupristin	1000–4000	0.241 ± 0.02	0.9974 ± 0.0018
Dalfopristin	480–1920	0.567 ± 0.04	0.9961 ± 0.0030

Table 2
Precision and accuracy data for the determination of quinupristin and dalfopristin by LC-MS/MS

Compound	Nominal concentration ng ml^{-1}	Calculated concentration ng ml^{-1}[a]	%RSD[b]	%DEV[c]
Quinupristin	1200	1252.9 ± 53.0	4.2	−1.2 +9.1
	1700	1752.6 ± 23.1	1.3	+1.2 +5.1
Dalfopristin	1000	976.0 ± 22.9	2.3	−5.6 +0.3
	2000	1979.9 ± 78.7	4.0	−7.5 +3.7

[a] Mean ± S.D. ($n = 7$).
[b] %RSD, % relative standard deviation.
[c] % DEV, % deviation from nominal value.

composition of the mobile phase and type of HPLC column were selected to achieve a rapid and optimum detection of the analytes. A mobile phase composed of acetonitrile–water 70:30 w/w ratio at flow rate 0.4 ml min^{-1} was appropriate to detect the parent mass ions of dalfopristin and quinupristin by +ESI. An HPLC XTerra™ MS, C18, 2.5 μm (2.1 mm i.d.) column was suitable for MS analysis. Under these conditions, dalfopristin and quinupristin were rapidly detected (> 2 min) without complete chromatographic resolution, as their detection was based on fragmentation properties rather than on chromatographic behaviors.

3.2. Linearity

Calibration curves were constructed by weighed linear regression of the peak area of dalfopristin or quinupristin using external standard method at MRM transition; $691.87 > 166.26$ or $1023.05 > 134.34$, respectively. The plots and calibration parameters such as, the slope and correlation coefficient, were determined by MASSLYNX quantify program. Linear plots over the concentration ranges 1000–4000 ng ml^{-1} (quinupristin) and 480–1920 ng ml^{-1} (dalfopristin) were determined. Based on peak area (PA) measurements, the regression equations of the calibration curves for quinupristin and dalfopristin were: $PA = 0.154x + 14.07$ ($r = 0.9989$) for quinupristin and $PA = 0.501x + -30.69$ ($r = 0.9945$) for dalfopristin, where x is the concentration (ng ml^{-1}). Mean values of the slope and correlation coefficient of 8 calibration curves of quinupristin and dalfopristin, were listed in Table 1. The %RSD values of the slope and regression coefficient of the calibration curves were in the ranges of 7.05–8.29% and 0.18–0.30%, respectively. The lowest limits of quantitation of quinupristin and dalfopristin were 1000–480 ng ml^{-1}, respectively (%RSD < 10%). The calibration data showed that quinupristin was quantified at relatively higher concentration compared to dalfopristin. The ratio of slopes of the calibration curves of quinupristin and dalfopristin was found to be ~0.425. The calculated value was in good agreement with the nominal composition

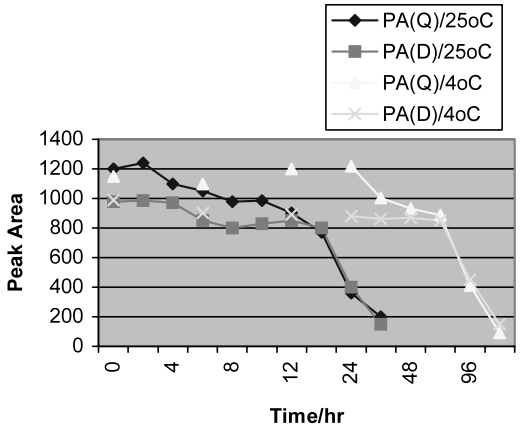

Fig. 7. Plot of peak area values of quinupristin (Q) and dalfopristin (D) versus time (h) determined by LC-MS/MS in stability studies at 4 and 25 °C.

ratio of the antimicrobial compounds quinupristin/dalfopristin in Synercid® injection (0.428).

3.3. Quality control samples

The precision and accuracy of the developed LC-MS/MS were assessed by determining %RSD and %DEV of the measured dalfopristin and quinupristin concentrations (Table 2). As indicated, the %RSD ranges were 2.3–4.0% for dalfopristin and 1.3–4.2% for quinupristin, whereas the %DEV ranges were (−7.5+3.7%) and (−1.2+9.1%) for dalfopristin and quinupristin, respectively. The data indicated that the developed method was accurate and reproducible for the quantitation of both constituents.

3.4. Selectivity, stability and recovery studies

The selectivity of the method was evaluated by examining the possible interferences from antimicrobial agents which might be intravenously administered with Synercid®. Cephalosporins such as cefotaxime and ceftriaxone exhibited no interference, as only a daughter ion related to target compound was selected using the specific MRM transition. Furthermore, to indicate the selectivity of LC-MS/MS method for the analyses of quinupristin and dalfopristin in Synercid®, stability studies were conducted in acetonitrile/water 70:30 w/w at room temperature (25 °C) and at low temperatures (4 and −20 °C). The studies showed that the stability of Synercid® solutions was influenced by the storage temperatures (Figs. 7 and 8). Accelerated stability studies revealed that the degradation of quinupristin and dalfopristin followed first-order kinetics. The calculated kinetic parameters for both components indicated slow degradation rates and longer degradation half-lives and shelf-lives at −20 °C (Table 3). According to the data obtained, it is recommended to dissolve the contents of injection in mixtures of aqueous and non-aqueous solvents and to store the prepared injections at −20 °C to minimize the degradation of the active constituents. Determination of quinupristin and dalfopristin contents in Synercid® injection was further considered to elucidate the robustness of the developed LC-MS/MS method. Mean recovery percentages of 99.5–103.8% and 97.8–99.0% were calculated for quinupristin and dalfopristin, respectively in Synercid® injection (Table 4).

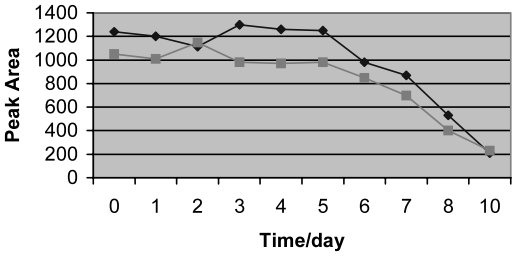

Fig. 8. Plot of peak area values of quinupristin (◆) and dalfopristin (■) versus time (day) determined by LC-MS/MS in stability studies at −20 °C.

4. Conclusion

The described HPLC-MRM method using tandem positive electrospray-MS/MS was proved to be highly selective for simultaneous analysis of quinupristin and dalfopristin in Synercid® injections. The developed method indicates a quinupristin/dalfopristin composition ratio of 0.425, which agrees with the nominal composition ratio 0.428 (30:70) of Synercid®. The run-cycle-time was ∼2 min, injection-to-injection, which was ideal

Table 3
Kinetic parameters for the degradation of quinupristin and dalfopristin at 25, 4 and −20 °C

Temperature	Quinupristin			Dalfopristin		
	K_{deg}	$t_{1/2}$[a]	t_{90}[b]	K_{deg}	$t_{1/2}$	t_{90}
25 °C[c]	0.052	13.32	2.02	0.049	14.14	2.14
4 °C[c]	0.020	34.65	5.25	0.014	49.50	7.50
−20 °C[d]	0.048	14.44	2.19	0.051	13.59	2.06

[a] $t_{1/2} = 0.693/K_{deg}$.
[b] $t_{90} = 0.105/K_{deg}$.
[c] K_{deg}: (h^{-1}), $t_{1/2}$ and t_{90} (h).
[d] K_{deg}: (day^{-1}), $t_{1/2}$ and t_{90} (day).

Table 4
Recovery percentages of quinupristin and dalfopristin from Synercid® injection using LC-MS/MS

Nominal Concentration (ng ml^{-1})	Mean %recovery ± S.D.
Quinupristin	
1200	103.3 ± 4.5 ($n = 5$)
2000	99.5 ± 5.5 ($n = 3$)
4000	103.8 ± 3.5 ($n = 5$)
Dalfopristin	
2000	97.8 ± 3.8 ($n = 5$)
2400	99.0 ± 2.3 ($n = 4$)

for QC purposes, for determination of quinupristin and dalfopristin contents of Synercid®. The method proved to be a stability-indicating, as it permits tracking of the stability and compatibility of the individual constituents at variable storage temperatures and in the presence of other antibiotics.

Acknowledgements

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