Rapid method development to study plasma stability of diverse pharmaceutical compounds using Rapid Resolution LC and triple quadrupole MS

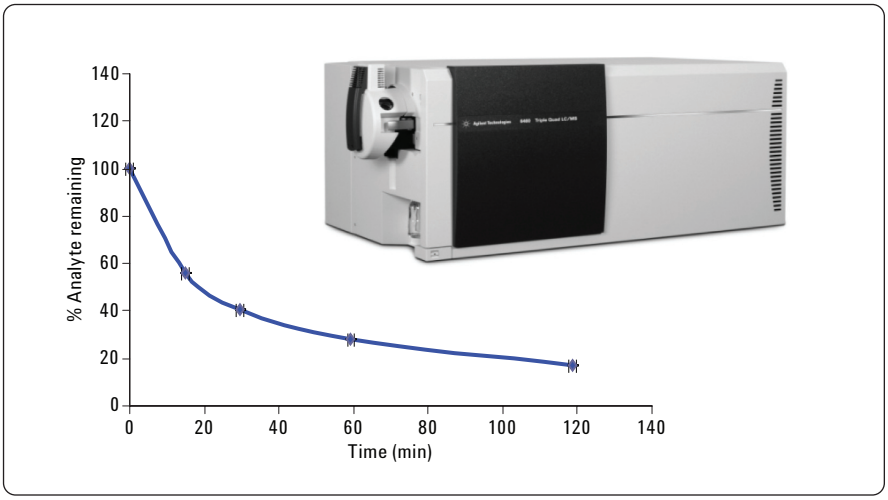
Application Note

Drug Discovery

Authors

Srividya Kailasam
Agilent Technologies
Bangalore
India

Kerry Frost
Cyprotex Discovery Limited



Abstract

This Application Note demonstrates the use of rapid resolution liquid chromatography in conjunction with tandem mass spectrometry to study the plasma stability of pharmaceutical compounds belonging to four different drug classes. The Agilent 1200 Series Rapid Resolution LC system (RRLC) provided fast separation of compounds and internal standard in all samples. The Agilent MassHunter Optimizer software identified compound-specific MS parameters such as the most abundant product ions, optimal fragmentor voltages and collision energies. Taken together, robust methods for a larger set of compounds could be developed within a short period of time.

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Introduction

Compounds with poor stability characteristics tend to have low bioavailability. Certain functional groups such as amides, esters or lactones make compounds favorable targets for plasma enzymes such as hydrolases and esterases. Knowledge of plasma stability profiles of New Chemical Entities (NCEs) helps in modifying the molecular structure to improve physicochemical properties and in selecting more suitable molecules for subsequent development.

In this study rapid resolution LC/MS-MS was used to study the plasma stability of ten pharmaceutical compounds. Four beta blockers (atenolol, labetalol, metoprolol and propranolol), one calcium channel blocker (verapamil), one serotonin receptor agonist (buspirone) and four antidepressants (nefazodone, nortriptyline, imipramine and trimipramine) were analyzed using reserpine as the internal standard. Eucatropine, an ophthalmic drug, was used as the control and analyzed using metoprolol as the internal standard.

Experimental

Chemicals

Atenolol, labetalol, metoprolol, propranolol, buspirone, nefazodone, nortriptyline, imipramine, trimiparmine, verapamil, eucatropine and reserpine were purchased from Sigma-Aldrich, Bangalore, India. HPLC-grade acetonitrile and methanol were purchased from Lab-Scan. Formic acid was purchased from Fluka.

Stock solutions and suitable dilutions of target analytes were prepared in methanol for method development.

Sample preparation

Plasma was adjusted to pH 7.4 with either 0.1 M hydrochloric acid or 0.1M sodium hydroxide. Test compounds were dissolved in DMSO to a final concentration of 10 mM and then diluted to 40 µM in DMSO. Incubations were carried at a test compound concentration of 1 µM with a final DMSO concentration of 2.5%. 195 µl of plasma was added to the wells of a 96- well plate before spiking them with the 5 µl of test compound.

The spiked plasma samples were incubated at 37 °C for 2 hrs. Reactions were terminated at 0, 15, 30, 60 and 120 min by adding 400 µl of methanol containing reserpine as an internal standard. Simultaneously, plasma samples containing eucatropine (control compound) were terminated by adding 400 µL of methanol containing metoprolol as an internal standard. In addition, matrix blanks were prepared by adding methanol solutions containing internal standards to plasma samples without any of the analytes or control compounds. The sample plate was centrifuged at 2500 rpm for 45 min at 4 °C and the supernatant was transferred to a fresh 96-well plate for analysis by LC/MS-MS.

Instrumentation

We used an Agilent 1200 Series Rapid Resolution LC system which included a micro vacuum degasser, binary pump, high-performance autosampler with thermostat and a thermostatted column compartment coupled to an Agilent 6460 Triple Quadrupole Mass Spectrometer. The mass spectrometer operated in ESI mode with Agilent's Jet Stream Technology. The Agilent MassHunter Workstation (version B.02.00) was used for data acquisition and MassHunter Quantitative analysis software (version B.01.04) was used for data analysis. We employed the Agilent MassHunter Optimizer software (B.02.00) for optimization of two important MS parameters: the fragmentor voltage and collision energies. Optimizer also provided the most abundant MRM transitions used in this study.

Method details

LC conditions used for all analytes and control are presented in Table 1.

Parameter	Detail	
Mobile phase	A: Water (0.1% Formic acid) B: Acetonitrile (0.1% Formic acid)	
Flow rate	0.5 mL/min	
Gradient conditions	0 min 0.2 min 0.3 - 0.5 min 0.6 - 1.2 min 1.25 - 1.5 min	20 % B 50 % B 70 % B 95 % B 20 % B
Run time	1.5 min	
Post time	1 min	
Injection volume	1 µl	
Needle wash	10 sec wash port with 1:1 methanol:water mix	
Column	Agilent Zorbax Eclipse XDB - C18, 2.1 × 50 mm, 1.8 µm particle size (p/n 927700-902)	
Column temperature	50 °C	

Table 1
LC Conditions.

Reserpine was used as the internal standard for the analytes under study and metoprolol was used as the internal standard for eucatropine which was the control for the study. A generic set of MS conditions as shown in Table 2 were applied for ionization of all compounds including the control and the two internal standards.

Parameter	Detail
Ionization mode	ESI-positive with Agilent Jet Stream Technology
Drying gas temperature	350 °C
Drying gas flow rate	10 L/min
Nebulization gas pressure	20 Psi
Capillary voltage	2000 V
Sheath gas temperature	400 °C
Sheath gas flow rate	11 L/min
Nozzle voltage	0 V
Q1 resolution	Unit
Q2 resolution	Unit

Table 2
MS conditions.

The MassHunter Optimizer identified compound-specific MS parameters such as optimal fragmentor voltages, most abundant product ions for the selected precursor ion, and associated collision energies. The MRM transitions used in the study are presented in Table 3. A 50-ms dwell time used for all transitions provided more than 50 data points across all peaks.

Compound	Empirical Formula	Precursor Ion	Product Ion(s) Quantifier, Qualifier	Fragmentor voltage (V)	Collision energy (eV) Quantifier, Qualifier
Atenolol	$C_{14}H_{22}N_2O_3$	267.2	145, 190	115	25, 13
Labetolol	$C_{19}H_{24}N_2O_3$	329.2	311.1, 294.1	105	5, 13
Metoprolol	$C_{15}H_{25}NO_3$	268.2	116.1, 74.1	115	13,17
Propranolol	$C_{16}H_{21}NO_2$	260.2	116.1,183	110	13,13
Buspirone	$C_{21}H_{31}N_5O_2$	386.3	122, 150	164	29,29
Verapamil	$C_{27}H_{38}N_2O_4$	455.3	165, 303.1	158	25, 21
Nefazodone	$C_{25}H_{32}ClN_5O_2$	470.2	274.1, 246.1	150	25,33
Nortriptyline	$C_{19}H_{21}N$	264.2	233.1, 90.9	80	10, 20
Imipramine	$C_{19}H_{24}N_2$	281.2	86.1, 58.1	101	13, 39
Trimipramine	$C_{20}H_{26}N_2$	295.2	100.1, 58.1	105	9,41
Eucatropine	$C_{17}H_{25}NO_3$	292.2	58.1	120	21
Reserpine	$C_{33}H_{40}N_2O_9$	609.38	195.1	150	41

Table 3
MS/MS parameters.

Data analyses

Five replicate injections were performed for each sample. The mean values of relative responses (analyte concentration/internal standard concentration) at different time points were calculated. The mean relative response at each time point was normalized with respect to mean relative response at 0 min (T_0). The normalized values (as percentages) were plotted against exposure time to obtain a plasma stability profile.

Results and discussion

This screening assay identifies any change in plasma concentration relative to the initial concentration at 0 min. Therefore, it is not necessary to determine the exact concentration in the samples. The percentage of parent remaining at each time point relative to the 0-min sample is calculated. A compound is considered stable in plasma if the percentages at each time point are within ± 15% of the 0-min sample.

Figure 1A shows two overlaid peaks corresponding to the quantifier and qualifier transitions of one of the target compounds, nortriptyline, along with a peak representing the transition for the internal standard, reserpine. Figure 2A shows MRM transition peaks of eucatropine and its internal standard, metoprolol. A short run time of 1.5 min enables fast analyses of multiple samples.

The selectivity of the technique can be seen in Figures 1B and 2B which are the MRM chromatograms of sample and control blanks respectively. The most abundant peak(s) in the two figures are of the two internal standards, reserpine and metoprolol (quantifier and qualifier) respectively. No significant peaks are seen for control or target compounds.

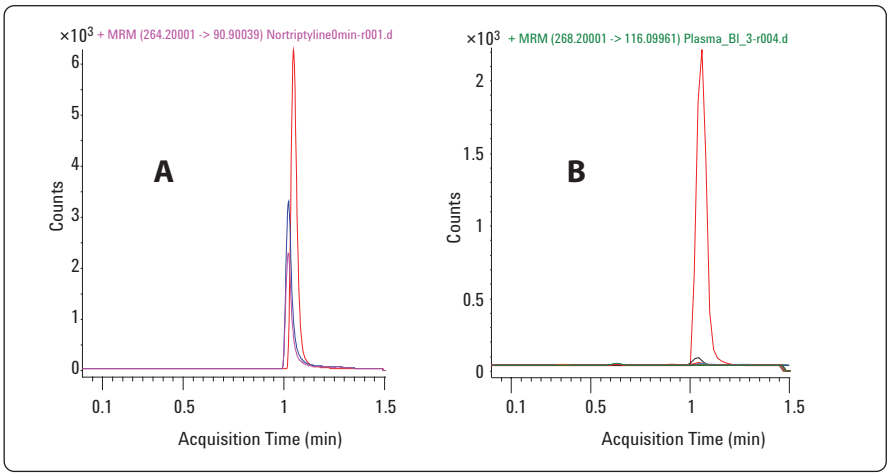

Figure 1A
MRM chromatogram of a study sample showing nortriptyline and IS (reserpine) peaks
Figure 1B
Plasma blank of samples containing only (IS) reserpine.

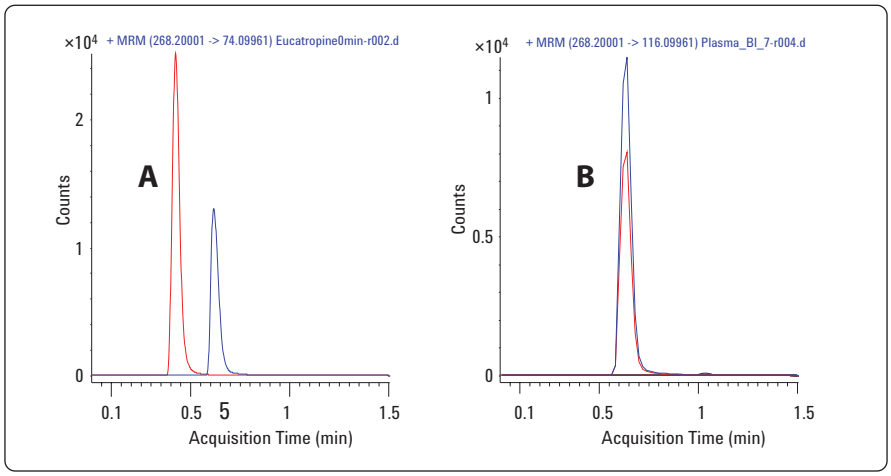

Figure 2A
MRM chromatogram of control showing eucatropine and (IS) metoprolol peak.
Figure 2B
Plasma blank of control containing only (IS) metoprolol.

Figure 3 depicts the plasma stability of the control compound eucatropine along with plasma stabilities of two of the target compounds. The developed method was able to successfully track the known stability characteristic of eucatropine [1]. Nortriptyline undergoes degradation in plasma, while metoprolol was found to be stable for up to two hours in plasma. Fluctuations in plasma concentrations after different contact times, despite excellent injection precision (CV <15%), was similar to what has been reported [2]. Deviation in concentration within ± 20% is acceptable for a screening assay. The plasma stabilities for all the target analytes and control compounds are shown in Table 4.

Compound	Plasma Stability
Atenolol	Stable
Labetolol	Stable
Metoprolol	Stable
Propranolol	Unstable
Buspirone	Stable
Verapamil	Stable
Nefazodone	Stable
Nortriptyline	Unstable
Imipramine	Stable
Trimipramine	Stable
Eucatropine	Unstable

Table 4
Plasma stability characteristics.

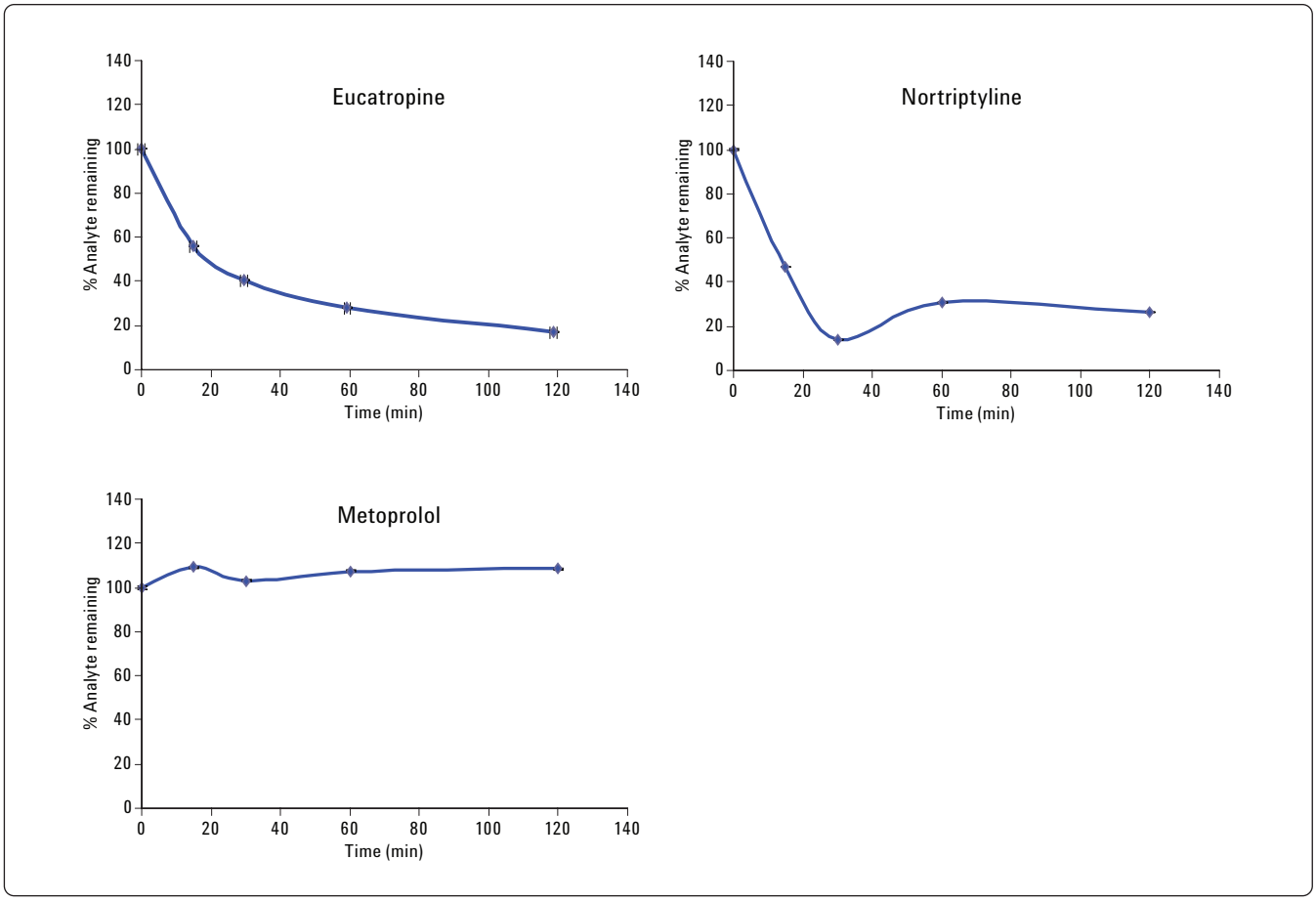

Figure 3
Stability curves for three analytes.

Conclusion

We developed an LC method along with a set of mass spectrometric conditions to assess plasma stability of ten pharmaceutical compounds. The MassHunter Optimizer software identified the most intense product ions for the selected precursor ion. The software also provided the most suitable fragmentor voltages and collision energies. Therefore, the mass spectrometric method could be developed quickly for a standard compound. We could show the suitability of the proposed method for estimating plasma stability of compounds belonging to different classes.

References

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October 1, 2009
Publication Number 5990-4603EN

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