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LC–MS–MS Analysis of Terfenadine and Its Metabolites in Rat Plasma Using Oasis® MCX

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The high performance liquid chromatography (HPLC) system used was a Waters Alliance 2795 HPLC system equipped with a sample chiller and an in-line solvent degasser. The analytical column was an XTerra® MS C18 column (2.1 mm × 30 mm, 3.5 µm). A Micromass Quattro Ultima triple quadrupole mass spectrometer was equipped with an electrospray source and set in positive acquisition using multiple reaction monitoring (MRM). Data acquisition and instrument control were powered by MassLynx™ chromatographic software (Micromass, Ltd., United Kingdom). The µElution SPE 96-well plate was packed with 2 mg of Oasis MCX.

Experimental Conditions

Sample preparation: Pipette 5 mL of raw uncentrifuged rat plasma (heparin as anticoagulant) into ten 10 mL scintillation vials for the calibration curve. Add mixture of terfenadine, terfenadine–alcool, and terfenadine–carboxylate at various concentrations to achieve a calibration from 0.5 ng/mL to 200 ng/mL. Add 100 µL of concentrated phosphoric acid to all vials. Condition the Oasis MCX with 200 µL of methanol and pull through with a positive or negative displacement. Repeat with 200 µL of water. Load 250 µL of spiked rat plasma in each well, followed by 250 µL of internal standard (protriptyline) in water at 10 ng/mL. Apply vacuum to dryness. Wash with 200 µL of water and 0.1 N HCl, followed by 200 µL of methanol. Elute with 25 µL of 40:60 acetonitrile–isopropanol and 5% ammonium hydroxide in a 350-µL or 1-mL collection plate. Dilute the eluent with 50 µL of water and inject 25 µL of the resulting solution onto the LC system.

Table I: Analysis of terfenadine and its metabolite in rat plasma using Oasis MCX

Drug	Concentration (ng/mL)	Average (n = 6)	RSD	CV (%)
Terfenadine	0.5	0.51	0.006	2.0
	1.0	0.96	0.044	4.6
	5.0	4.99	0.240	4.8
	10.0	10.11	0.46	1.8
	20.0	19.26	0.355	1.8
	50.0	54.05	1.96	3.6
	100.00	103.25	3.36	3.2
	200.00	197.18	1.82	0.9
Terfenadine–alcool	0.5	0.494	0.010	2.3
	2.0	1.935	0.082	4.2
	5.0	5.05	0.246	4.8
	10.0	10.12	0.454	4.4
	25.0	24.82	1.157	4.6
	50.0	50.51	2.22	4.3
	100.0	101.69	3.03	2.9
	200.0	196.39	4.12	2.1
Terfenadine–carboxylate	0.5	0.51	0.0075	1.4
	1.0	0.975	0.038	3.9
	5.0	5.192	0.254	4.9
	10.0	10.41	0.565	35.4
	20.0	19.80	0.722	3.6
	25.0	24.66	0.556	2.2
	50.0	51.08	1.72	3.3
	100.0	102.05	2.95	2.8
	200.0	196.55	7.40	3.7

Chromatographic conditions: The analytical column was an XTerra MS C18 column (2.1 mm × 30 mm, 3.5 µm) used with a gradient mobile phase consisting of 0.1 M ammonium formate at pH 9.5 in water (line A) and methanol (line B). Four compounds were separated in 4 min using gradient conditions at 0.4 mL/min with an injection volume of 25 µL. The gradient starts at 5% organic and ramps at 95% organic in 1 min, stays at 95% organic for 1 min more, drops to the original condition in 30 s, and reconditions for 2.5 min. The Quattro Ultima triple quadrupole system was set for analysis with electrospray in positive mode using MRM. The quantitation was performed on the MRM transition 472.2 → 436.3 for terfenadine, 502.2 → 466.2 for terfenadine–carboxylate, 488.2 → 452.2 for terfenadine–alcool and 263.9 → 190.8 for the internal standard (protriptyline).

Results and Conclusion

The average recovery of terfenadine and its metabolite (n = 6), shown in Table I, was 85% and higher with an average coefficient of variation (CV) of 5%. In this situation, sub-nanograms per milliliter LOQ was reached with a simple mixed-mode generic method and gave higher sensitivity. The two-step cleanup in the Oasis MCX protocol removes more interferences than the generic Oasis HLB.

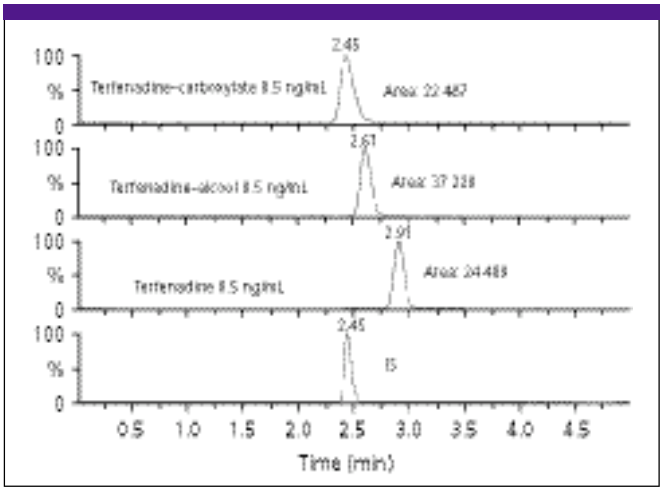

Figure 1: Analysis of terfenadine in rat plasma using Oasis MCX.

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