A novel LC-TQ-MS/MS method for quantifying Mefenamic acid-NDSRI (N-Nitroso drug substance-related impurity) in Mefenamic acid tablet and pediatric suspension dosage forms: A comparative study with cost-effective white, green, and blue UPLC method

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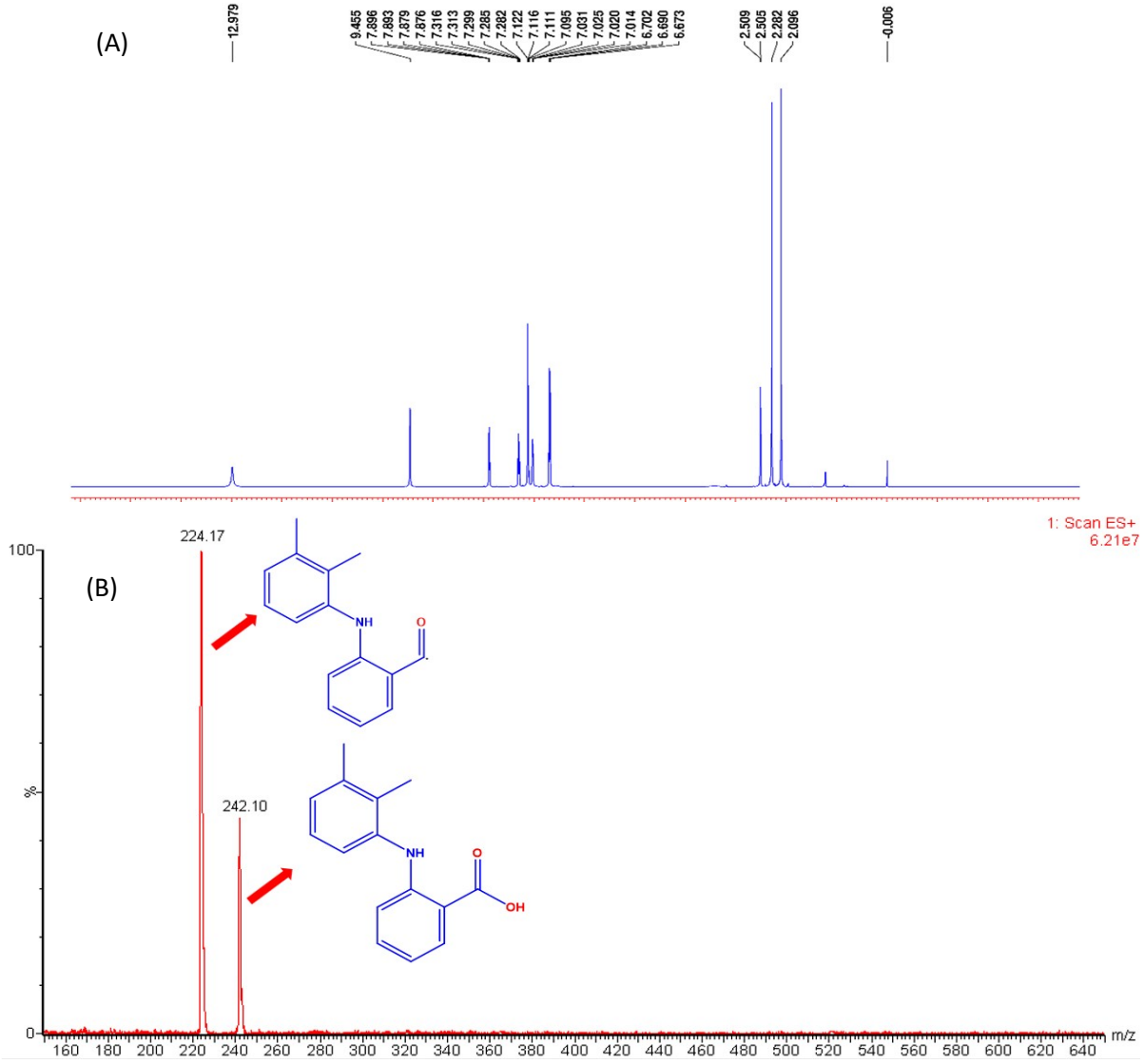


Fig. S1 (A)^{1}H-NMR and (B) mass spectra of MFA

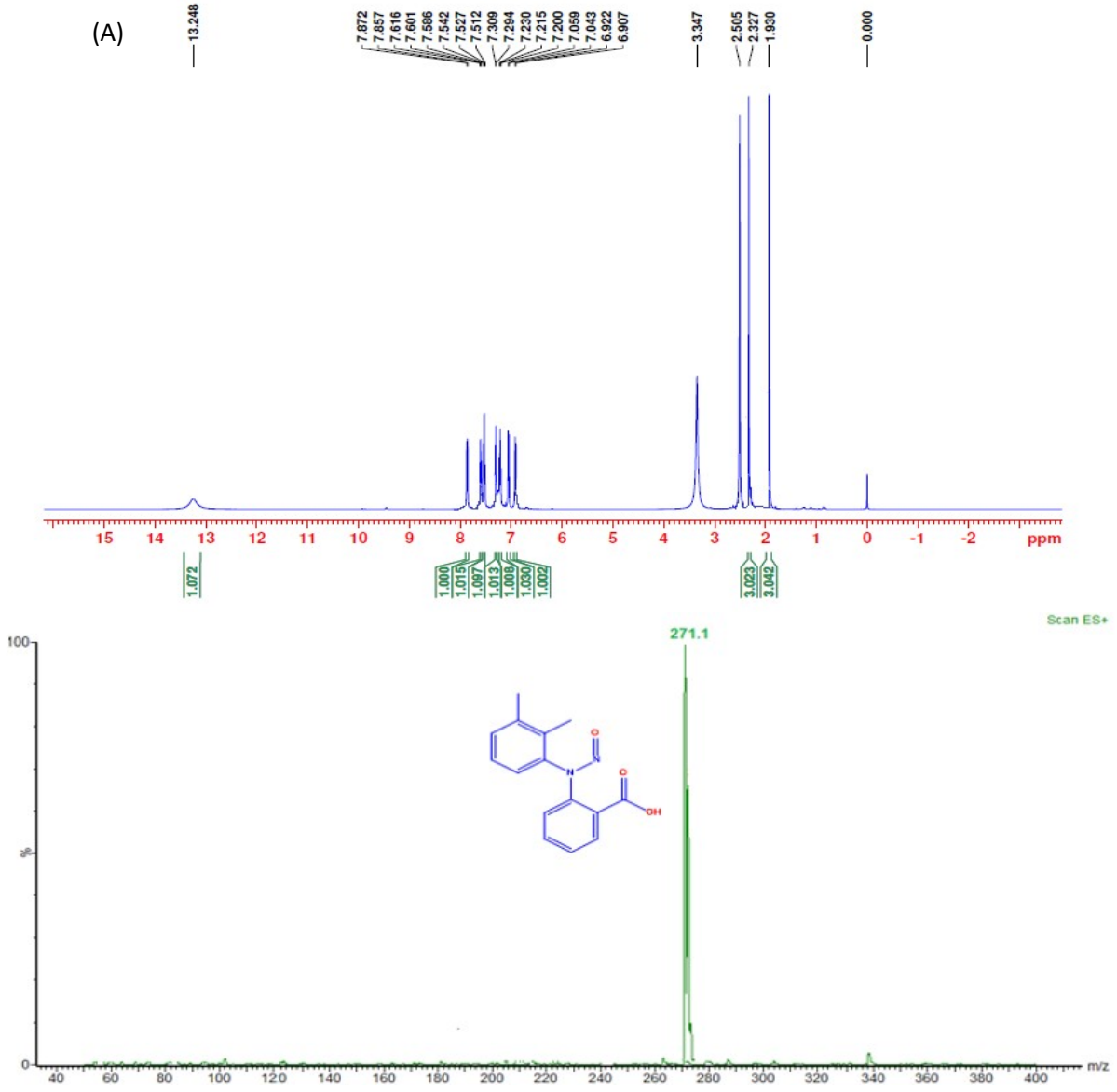


Fig. S2 (A)^{1}H-NMR and (B) mass spectra of N-MFA

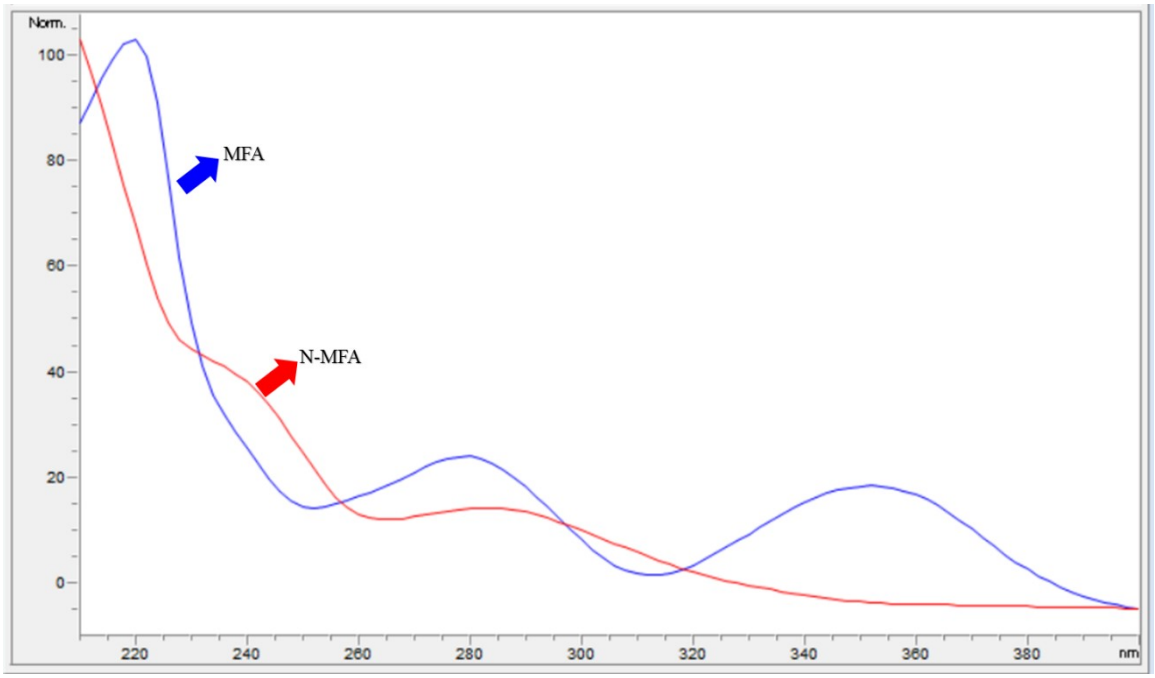


Fig. S3. Overlay UV spectra of MFA and N-MFA

Fig. S4 MRM chromatogram of N-MFA quantifier-qualifier

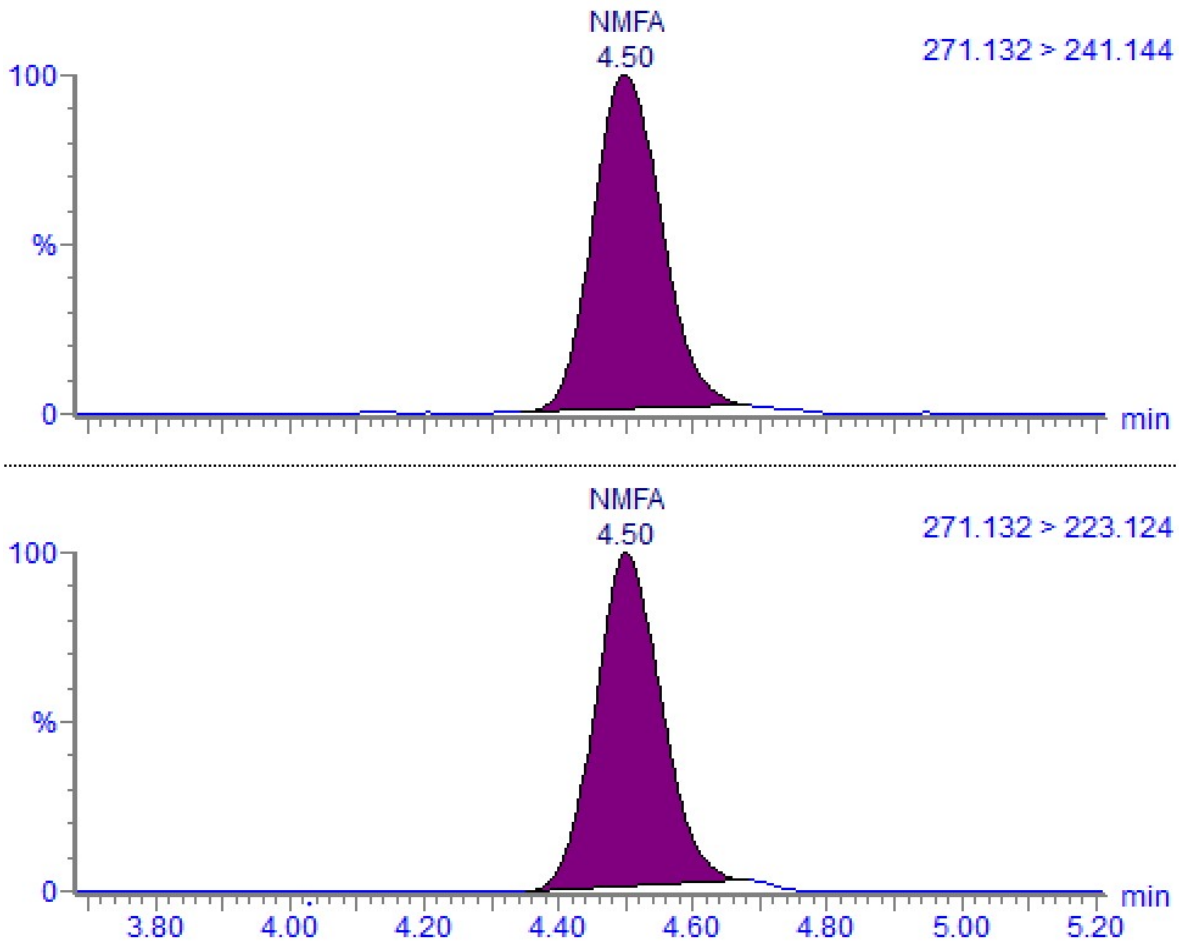
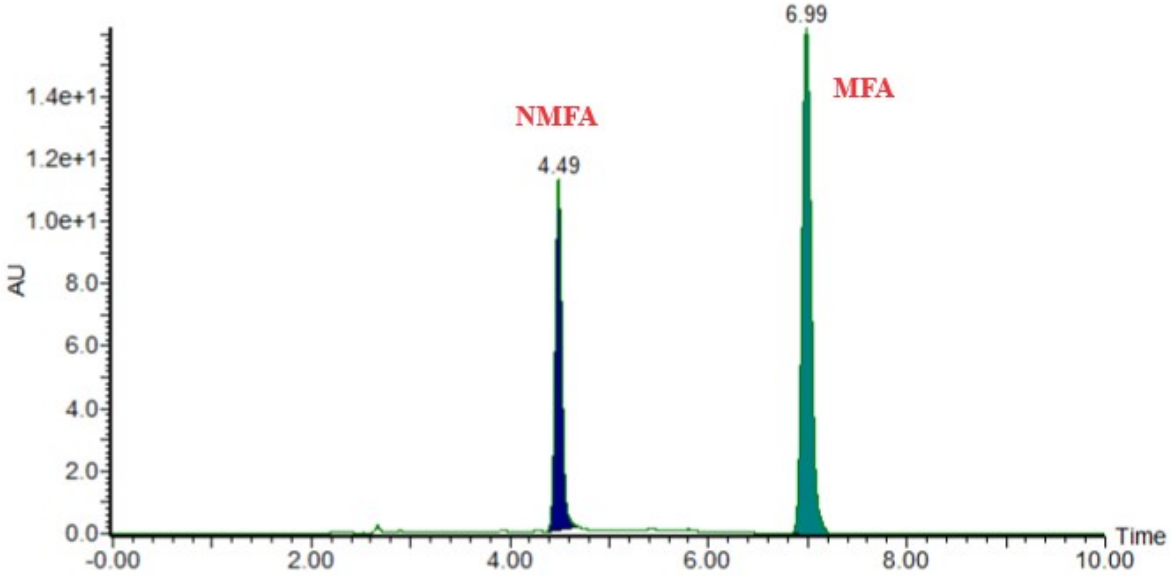


Fig. S5. Typical system suitability test chromatogram

Fig. 6 The whiteness assessed results for the proposed (A) LC-TQ-MS/MS, and (B) UPLC



method using the RGB12 Algorithm tool and the 12 principles of the WAC evaluation

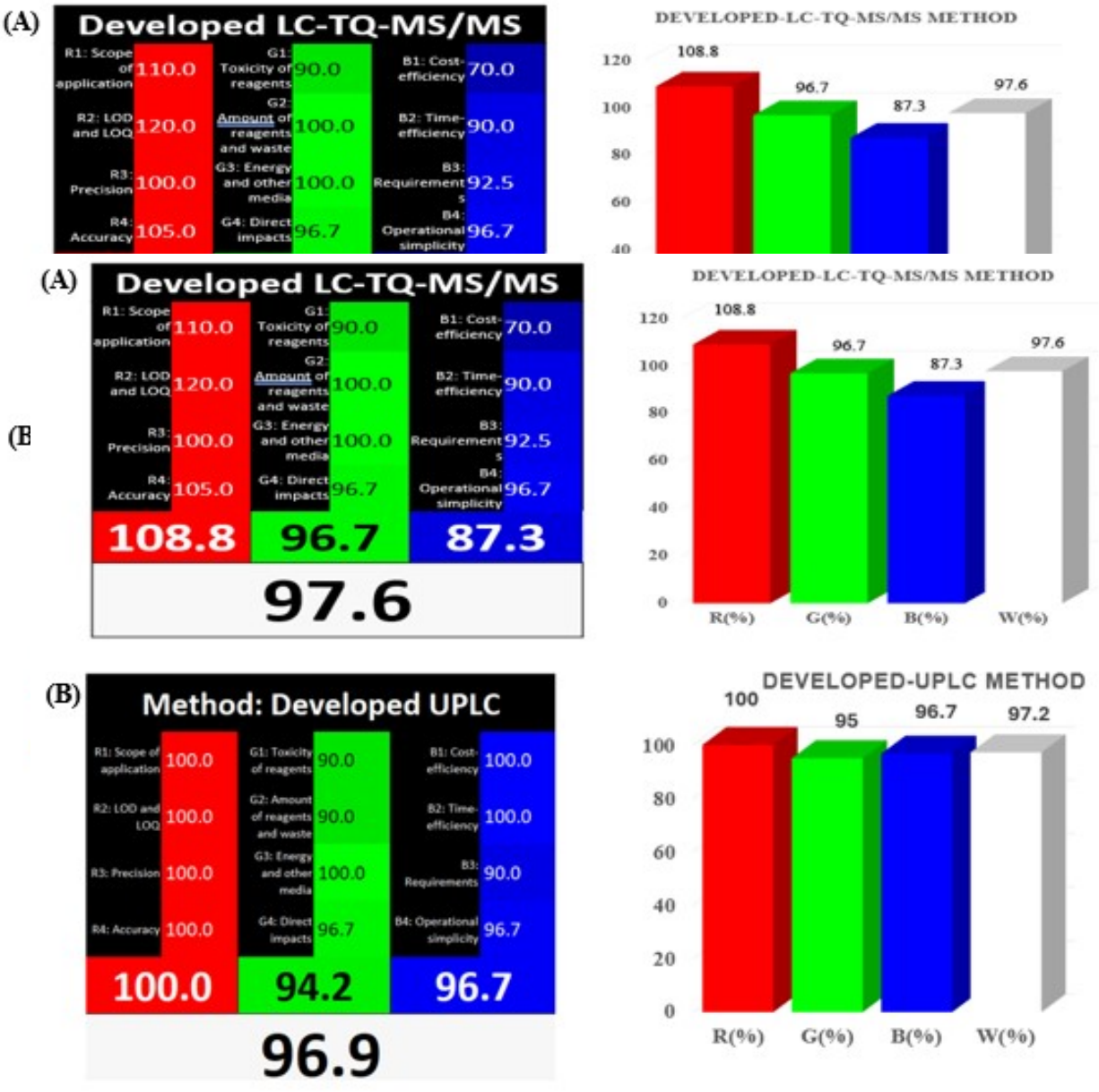


Table S1

The results of the mobile phase and column selection experiments

Mobile phase	Column/Column temp. (°C)	Flow rate (mL/min)/Gradient program	Observation	Interference
A: 10 mM ammonium acetate Buffer, B: Acetonitrile	Hypersil BDS C18 (150 × 2.1) mm, 5 µm/40 °C	0.8 mL/min/(T(min)/%B: 0.0/10, 2.0/10, 8.0/90, 13.0/90, 13.1/10, & 15.0/10)	Improper peak shape was observed	Not acceptable
A: 10 mM ammonium formate Buffer, B: Acetonitrile	Symmetry C18 (100 × 4.6) mm, 3.5 µm)/30 °C	0.8 mL/min/(T(min)/%B: 0.0/10, 2.0/10, 8.0/90, 13.0/90, 13.1/10, & 15.0/10)	Improper peak shape and peaks are coeluted	Not acceptable
A: 0.1% formic acid Buffer, B: Acetonitrile	Symmetry C18 (100 × 4.6) mm, 3.5 µm /40 °C	0.8 mL/min/(T(min)/%B: 0.0/10, 2.0/10, 4.0/90, 10.0/90, 10.1/10, & 12.0/10)	Poor resolution b/n MFA and N-MFA (<1.5)	Not acceptable
A: 0.1% formic acid Buffer, B: Acetonitrile	Hypersil BDS C18 (150 × 2.1) mm, 5 µm/40 °C	0.8 mL/min/(T(min)/%B: 0.0/10, 2.0/10, 8.0/90, 13.0/90, 13.1/10, & 15.0/10)	Improper peak shape and Poor resolution b/n MFA and NMFA (<1.5)	Not acceptable
A: 0.1% formic acid Buffer, B: Acetonitrile	XBridge BEH Shield RP18 Column (150 × 3.0 mm, 3.5 µm)/40 °C	0.8 mL/min/(T(min)/%B: 0.0/10, 2.0/10, 4.0/90, 10.0/90, 10.1/10, & 12.0/10)	Resolution b/n MFA and N-MFA	Acceptable and tried to reduce the run time
A: 0.1% formic Buffer, B: Acetonitrile	XBridge BEH Shield RP18 Column (150 × 3.0 mm, 3.5 µm)/40 °C	0.7 mL/min/(T(min)/%B: 0.0/10, 2.0/10, 4.0/80, 6.0/80, 6.1/30, and 8/10	Resolution b/n MFA and N-MFA (10.35)	Acceptable (Current method)

Table S2
Summarized results of the robustness study

Altered condition	Change	NMFA content (*ppm*)		% absolute difference with respect to the nominal condition	
		LC-TQ-MS/MS	UPLC	LC-TQ-MS/MS	UPLC
Concentration of formic acid (%)	0.09	62.55	62.50	0.06	0.06
	0.1	**62.59**	**62.54**	-	-
	0.11	62.52	62.56	0.11	0.03
Flow rate (mL/min)	0.8	62.49	62.49	0.16	0.08
	1.0	**62.59**	**62.5**4	-	-
	1.2	62.52	62.52	0.11	0.03
Column temp (°C)	38.0	62.53	62.50	0.10	0.06
	40.0	**62.59**	**62.54**	-	-
	42.0	62.51	62.51	0.13	0.05
Wavelength (nm)	228	62.52	62.52	0.11	0.03
	230	**62.59**	**62.54**	-	-
	232	62.57	62.57	0.03	0.05
Deslovation gas flow (L/Hr)	950	62.52		0.11	
	900	**62.59**		-	
	850	62.57		0.03	
Deslovation gas temp. (°C)	500	62.52		0.11	
	450	**62.59**		-	
	400	62.57		0.03	

Table S3
Summary of N-MFA content in MFA tablet and Pediatric suspension formulations

Brand	NMFA content (ppm)	
	LC-MS/MS	UPLC
Brand-A	3.36	< LOQ
	3.38	< LOQ
	3.29	< LOQ
Brand-B	4.64	4.21
	4.27	4.51
	4.38	4.35
Brand-C	3.31	< LOQ
	3.28	< LOQ
	3.19	< LOQ
Pediatric suspension	5.59	5.47
	5.70	5.81
	5.48	5.55

Table S4

Analytical eco-scale penalty points

Principle/ Reagents/Chemicals	Penalty points	
	LC-MS/MS	UPLC
Formic acid	6	6
Acetonitrile	8	8
LC-MS/UPLC	3	1
Occupational hazard	3	3
Sample storage (RT)	0	0
Sonicator	1	1
Waste	5	5
Waste Management	1	1
The sum of total penalty points	27	25
Analytical Eco-Scale	**73**	**75**

Table S5

GAPI and AGREE principles, method conditions, and score values

GAPI				AGREE			
Pictogram Name	Sub Part no and name	Method Condition		Principle	Method Condition	Principle Score	
		LC-TQ-MS/MS	UPLC			LC-TQ-MS/MS	UPLC
Sample sourcing	1. Collection	Off-Line	Off-Line	1. Sample treatment	Off-line analysis	0.48	0.48
	2. Preservation	None	None	2. Sample Amount	0.5 g	0.75	0.75
	3. Transport	None	None	3. Device Positioning	Off-line	0.0	0.0
	4. Storage	Normal Condition	Normal Condition	4. Sample Prep. Stages	3 or fewer	1.0	1.0
Method type	5. Direct/Indirect	Simple Preparation	Simple Preparation	5. Automation, miniaturization	Automatic, none or miniaturized	0.5	0.5
Sample preparation	6. Scale of extraction	nano	macro	6. Derivatization	NA	1.0	1.0
	7. Solvents/Reagents	Non- Green	Non- Green	7. Waste	15 mL	0.29	0.29
	8. Additional treatments	None	None	8. Analysis Throughput	1	1.0	1.0
Reagents and chemicals used	9. Amount	15 mL	15 mL	9. Energy consumption	LC-MS/UPLC	0.0	1.0
	10. NFPA health hazard value	Acetonitrile:2, Orthophosphoric acid (OPA):3	Acetonitrile:2, Orthophosphoric acid (OPA):3	10. Source of reagents	No reagents	1.0	1.0
	11. NFPA flammability value	Acetonitrile:3, Orthophosphoric acid (OPA):0	Acetonitrile:3, Orthophosphoric acid (OPA):0	11. Toxicity	18 mL	0.8	0.8
Instrumentation	12. Energy usage	<0.1 KWh per sample	<0.1 KWh per sample	12. Operator safety	Highly flammable	0.8	0.8
	13. Occupational hazard	Vapours to atmosphere	Vapours to atmosphere	**Method AGREE Score**		**0.63**	**0.68**
	14. Waste	>10 mL	>10 mL				
	15.	No Treatment	No Treatment				
Symbol	O-Present	Qualitative and Quantitative	Qualitative and Quantitative				