Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Table 1. Stability results of the studied drugs in spiked milk at different conditions for TLC-densitometeric method.

Oxytetracycline		
Concentration of the analyte (µg /band)	**Three freeze thaw cycles (%Recovery ± %RSD) ***	**Bench top stability (%Recovery ± %RSD)***
0.50	96.70±0.82	96.48±2.52
6.00	100.97±1.06	101.25±0.57
9.00	99.37±1.40	100.18±0.18
Mean ± RSD	**99.01±1.09**	**99.30±1.093**
Bromhexine		
Concentration of the analyte (µg /band)	**Three freeze thaw cycles (%Recovery ± %RSD) ***	**Bench top stability (%Recovery ± %RSD) ***
0.10	98.28±2.82	94.41±2.55
1.20	101.20±1.86	98.15±3.01
1.80	96.16±1.37	98.10±2.89
Mean ± RSD	**98.55±2.22**	**96.89±2.82**

*Average of 5 determinations

Supplementary Table 2. Stability results of the studied drugs in spiked milk at different conditions for RP-HPLC method.

Oxytetracycline		
Concentration of the analyte (µg/mL**)**	**Three freeze thaw cycles (%Recovery ± %RSD) ***	**Bench top stability (%Recovery ± %RSD) ***
0.10	99.94±2.30	99.64±0.92
10.00	101.93±1.49	100.82±0.44
30.00	97.52±3.12	98.56±0.84
Mean±RSD	**99.80±2.30**	**99.67±0.73**
Bromhexine		
Concentration of the analyte (µg/mL**)**	**Three freeze thaw cycles (%Recovery ± %RSD) ***	**Bench top stability (%Recovery ± %RSD) ***
0.20	98.78±1.96	100.16±2.28
10.00	102.60±1.12	102.32±1.21
20.00	101.52±1.95	101.66±1.44
Mean±RSD	**100.97±1.68**	**101.38±1.64**

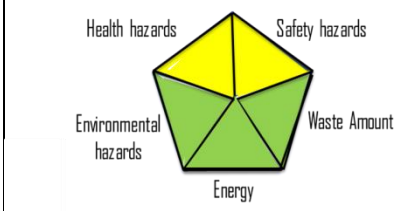
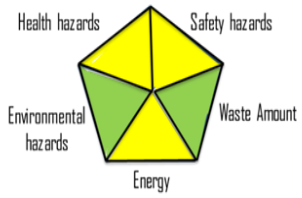
*Average of 5 determinations

Supplementary Table 3. NEMI metric system for the assessment of of TLC-densitometeric method , RP-HPLC method and the reported method.

	PBT reagents	Non PBT reagents	Hazardous reagents (RCRA included solvents)	Nonhazardous (RCRA non included solvents)	corrosive	waste	Pictogram
TLC	Methanol-Dichloromethane	Acetic acid-EDTA	Methanol-Acetic acid	EDTA-Dichloromethane	2<pH < 12	Generated waste =6.05 ml < 50mL	
HPLC	-----	Ethanol-Acetic acid-water-EDTA	Acetic acid	Ethanol-water	2<pH < 12	Generated waste =13.7ml < 50mL	
Reported HPLC [34]	Methanol acetonitrile	Trifluroacetic acid	Trifluroacetic acid acetonitrile	water	2<PH < 12	Generated waste =22ml < 50ml	

Supplementary Table 4. Modified NEMI metric system for the assessment of TLC-densitometeric , RP-HPLC and the reported method.

	TLC			HPLC			Reported HPLC [34]		
Category	**Green**	**Yellow**	**Red**	**Green**	**Yellow**	**Red**	**Green**	**Yellow**	**Red**
Health Hazard According to NFPA health hazards score : -Green :0-1 -Yellow:2-3 -Red: 4-5		**Acetic acid=3 Methylene chloride=2 Methanol= 2 EDTA= 1**			**Acetic acid=3 Ethanol= 2 EDTA=1**			**trifluro acetic acid=1 Acetonitrile=2 Methanol=2**	
Safety hazard According to NFPA flammability hazards score : -Green :0-1 -Yellow:2-3 -Red: 4-5		**Acetic acid=2 Methylene chloride=1 Methanol= 3 EDTA= 0**			**Acetic acid=2 Ethanol= 3 EDTA=0**			**trifluro acetic acid=0 Acetonitrile= 3 Methanol= 3**	

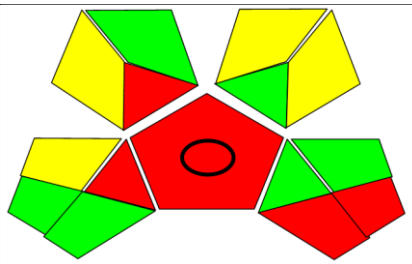
Environmental hazard -Green: If the Environmental hazard is less than 50 g. -Yellow: If the Environmental hazard is ranged from 50-250g -Red: If the Environmental hazard is higher than 250g.	**Environmental hazard is less than 50**			**Environmental hazard is less than 50**			**Environmental hazard is less than 50**		
Energy	**Instrumental method TLC**				**Instrumental method HPLC <1.5**			**Instrumental method HPLC <1.5**	
Waste amount The total waste was less than 50 g or mL for one sample analysis.	**Less than 50 g**			**Less than 50 g**			**Less than 50 g**		
Pictograms									

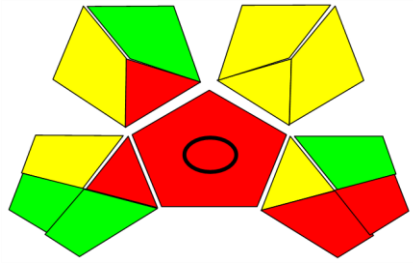
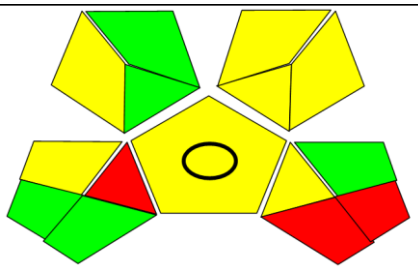
Supplementary Table 5. GAPI metric system for the assessment of TLC-densitometeric , RP-HPLC and the HPLC reported methods.

Sample Preparation								
	Collection	preservation	transport	storage	Type of method	Scale of extraction	Solvents/reagents used	Additional treatments
TLC	Off-line	None	None	Under normal conditions	Extraction required	Macro-extraction	Green solvents/ reagents	None
HPLC	Off-line	None	None	Under normal conditions	Extraction required	Macro-extraction	Green solvents/ reagents	None
Reported method [34]	Off-line	None	None	Under normal conditions	Simple procedures	-	Non-green solvents/ reagents	None

Reagents and Solvents			
	Amount	Health Hazards	Safety Hazards
TLC	< 10 mL (< 10 g)	Moderately toxic; could cause temporary incapacitation; NFPA = 2 or 3.	Highest NFPA flammability or instability score = 2 or 3, or a special hazard is used.
HPLC	10-100 mL (10-100 g)	Moderately toxic; could cause temporary incapacitation; NFPA = 2 or 3.	Highest NFPA flammability or instability score = 2 or 3, or a special hazard is used.
Reported method [34]	10-100 mL (10-100 g)	Moderately toxic; could cause temporary incapacitation; NFPA = 2 or 3.	Highest NFPA flammability or instability score = 2 or 3, or a special hazard is used.

Instrumentation				
	Energy	Occupational Hazards	Waste	Waste Treatment
TLC	<= 0.1 kWh per sample	Hermetic sealing of the analytical process	< 10 mL (>10 g)	No treatment
HPLC	<= 1.5 kWh per sample	Hermetic sealing of the analytical process	> 10 mL (>10 g)	No treatment
Reported method [34]	<= 1.5 kWh per sample	Hermetic sealing of the analytical process	> 10 mL (>10 g)	No treatment

Pictograms	
TLC	

HPLC	
Reported method [34]	

Supplementary Table 6. AGREE metric system for the assessment of TLC , TLC-densitometeric , RP-HPLC and the HPLC reported methods.

Parameters	TLC	RP-HPLC	Reported HPLC method [34]
Principle 1. Direct Analytical Techniques Should Be Applied to Avoid Sample Treatment. (Sampling Procedure)	Off-line Procedure	Off-line Procedure	Off-line Procedure
Principle 2. Minimal Sample Size and Minimal Number of Samples Are Goals. (Amount of sample in either gm or ml)	0.5 ml	0.5ml	0.5
Principle 3. In Situ Measurements Should Be Performed. (Positioning of analytical device)	Off-line Procedure	Off-line Procedure	Off-line Procedure
Principle 4. Integration of Analytical Processes and Operations Saves Energy and Reduces the Use of Reagents. (sample steps preparation)	3	3	Fewer than 3
Principle 5. Automated and Miniaturized Methods Should Be Selected. (Degree of automation and sample preparation)	Semi-automated-none or miniaturized	Semi-automated-none or miniaturized	Semi-automated-none or miniaturized
Principle 6. Derivatization Should Be Avoided. (Derivatization agents)	None	None	None
Principle 7. Generation of a Large Volume of Analytical Waste Should Be Avoided and Proper Management of Analytical Waste Should Be Provided. (amount of waste in gm or ml)	6.05 mL	13.7 mL	22 mL
Principle 8. Multianalyte or Multiparameter Methods Are Preferred versus Methods Using One Analyte at a Time. (number of analytes analysed in single run / samples analysed per hour)	3/20	3/5.45	3/5

Principle 9. The Use of Energy Should Be Minimized.(most energy-intensive technique used in method)	0.15	0.44	0.44
Principle 10. Reagents Obtained from Renewable Source Should Be Preferred. (Types of reagents)	Some reagents are bio-based	Some reagents are bio-based	all reagents are bio-based
Principle 11. Toxic Reagents Should Be Eliminated or Replaced.(Involvement of toxic reagents - number of toxic reagents)	Yes – 3.65 (acetic acid - methanol)	Yes – 3.65 (acetic acid and ethanol)	Yes – 12.8 (methanol and acetonitrile)
Principle 12. The Safety of the Operator Should Be Increased. (Threats which are not avoided)	Toxic to aquatic life (acetic acid) Highly flammable (methanol and methylene chloride) Corrosive (acetic acid-NaOH)	Toxic to aquatic life (acetic acid) Highly flammable(ethanol) Corrosive (acetic acid)	Toxic to aquatic life (trifluroacetic acid) Highly flammable(methanol-acetonitrile) Corrosive (trifluroacetic acid)
Clock like AGREE graph			

Supplementary Table 7. Eco-scale metric system for the assessment of TLC-densitometeric , RP-HPLC and the HPLC reported methods.

TLC								
Parameters	Reagents				Instrument			
	Methanol	Methylene chloride	Acetic acid	EDTA	Energy	Occupational hazards	wastes	
							Waste generated	Waste treatment
Consumed volume/sample (ml)	3.8mL	0.2mL	0.6 (Extraction) +0.2 (System)	0.9mL(extraction)	<0.1 kWh per sample	Analytical process hermetization	<10 mL	No waste treatment
Subtotal PP solvent>1ml=1PP solvent 10-100=2PP Solvent > 100=3pp	1	1	1	1				
Signal word(Danger = 2PP , Warning = 1PP)	2	1	2	2				
No. of pictogram	3	2	2	1				
PP of solvent = subtotal PP x signal words PP x no.pictograms	6	2	4	2	0 PP	0 PP	1 PP	**3**PP
Penalty points summation	**14**				**4**			
Total Penalty points	18							
Analytical Eco-scale score = 100-18 = 82								

RP-HPLC							
Parameters	Reagents			Instrument			
	Ethanol	Acetic acid	EDTA	Energy	Occupational hazard	waste	
						Waste generated	Waste treatment
Consumed volume = run time x flow rate x solvent percentage in mobile phase	5.3	0.6(extraction)+2.31(per sample)	0.9	≤1.5 kWh per sample	Analytical process hermetization	>10 mL	No waste treatment
Subtotal PP (solvent < 10ml)	1	1	1				
Signal word (Danger = 2PP , Warning = 1PP)	2	2	2				
No. of pictogram	2	2	1				
PP of solvent = subtotal PP x signal words PP x no.pictograms	4	4	2	1PP	0 PP	5 PP	3PP
Penalty points summation	**10**			**9**			
Total Penalty points	19						
Analytical Eco-scale score = 100-23 = 81							

Reported HPLC [34]							
Parameters	Reagents			Instrument			
	methanol	Trifluroacetic acid 0.1%	Acetonitrile	Energy	Occupational hazard	waste	
						Waste generated	Waste treatment
Consumed volume = run time x flow rate x solvent percentage in mobile phase	10	0.01	2.8	≤1.5 kWh per sample	Analytical process hermetization	>10 mL	No waste treatment
Subtotal PP (solvent < 10ml)	1	1	1				
Signal word (Danger = 2PP , Warning = 1PP)	2	2	2				
No. of pictogram	2	3	2				
PP of solvent = subtotal PP x signal words PP x no.pictograms	4	6	4	1PP	0 PP	5	3
Penalty points summation	**14**			**9**			
Total Penalty points	23						
Analytical Eco-scale score = 100-22 = 77							