

# Eco-Conscious Chromatographic Determination of Anti-depressant Free-Dose Combination in the Presence of Related Impurities.

Naglaa A. Kabil<sup>a</sup>, Hawaa M. Khalil<sup>a</sup>, Hind E. El Ghwas<sup>b</sup>, Shymaa Bibars<sup>b</sup>, and Sara Abdulwahab<sup>a\*</sup>

<sup>a</sup> Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

<sup>b</sup> Department of Analytical Chemistry, Egyptian Drug Authority (EDA), 51 Wezaret El-Zeera St, P. O. Box 29, Giza, Egypt

\*Corresponding Author

email: smanees@zu.edu.eg

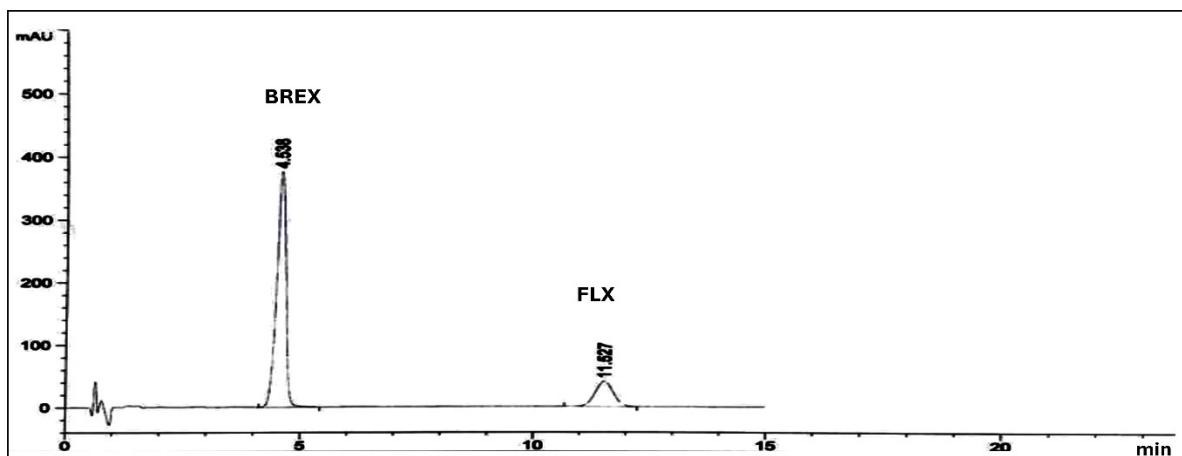


Figure S1: A typical LC chromatogram of the simultaneous determination of [1] BREX and [2] FLX in their free-dosage combination.

**Table (S1): Robustness study of the proposed RP-LC method for the simultaneous determination of BREX and FLX**

Parameters	Conditions	Area		Tailing Factor		Theoretical plate		Resolution	
		BREX	FLX	BREX	FLX	BREX	FLX	BREX	FLX
Wavelength (nm)	219	5032.637	1072.721	1.00	1.00	2554	3993	6.15	12.78
	220	5066.394	1108.548	0.97	0.98	2489	3944	6.20	12.69
	221	4924.453	1109.294	<b>0.97</b>	<b>0.99</b>	2566	3918	6.15	12.73
<i>Mean</i>		<b>5007.83</b>	<b>1096.85</b>	<b>0.98</b>	<b>0.99</b>	<b>2536.33</b>	<b>3951.67</b>	<b>6.17</b>	<b>12.73</b>
<i>RSD</i>		<b>1.48</b>	<b>1.91</b>	<b>1.77</b>	<b>1.01</b>	<b>1.63</b>	<b>0.96</b>	<b>0.47</b>	<b>0.35</b>
Temperature (°C)	49	5137.353	1100.192	0.98	0.99	2600	4042	6.25	12.99
	50	5178.249	1119.289	0.96	0.97	2571	3945	6.25	12.78
	51	5185.747	1121.879	0.97	0.96	2630	4099	6.35	12.99
<i>Mean</i>		<b>5167.12</b>	<b>1113.79</b>	<b>0.97</b>	<b>0.97</b>	<b>2600.33</b>	<b>4028.67</b>	<b>6.28</b>	<b>12.92</b>
<i>RSD</i>		<b>0.50</b>	<b>1.06</b>	<b>1.03</b>	<b>1.57</b>	<b>1.13</b>	<b>1.93</b>	<b>0.92</b>	<b>0.94</b>
Flow rate (mLmin <sup>-1</sup> )	1.4	5053.386	1096.307	0.95	0.97	2598	3998	6.2	12.62
	1.5	5114.053	1093.854	0.96	0.96	2548	3995	6.25	12.76
	1.6	5015.103	1095.994	0.95	0.97	2497	3868	6.2	12.62
<i>Mean</i>		<b>5060.85</b>	<b>1095.39</b>	<b>0.95</b>	<b>0.97</b>	<b>2547.67</b>	<b>3953.67</b>	<b>6.22</b>	<b>12.67</b>
<i>RSD</i>		<b>0.99</b>	<b>0.12</b>	<b>0.61</b>	<b>0.60</b>	<b>1.98</b>	<b>1.88</b>	<b>0.46</b>	<b>0.64</b>
Injection volume (μL)	19	4864.657	3955	0.98	1.01	2609	3955	6.25	12.82
	20	4922.914	3983	0.97	0.99	2630	3983	6.3	12.91
	21	4994.918	3971	0.97	1.00	2696	3971	6.2	12.81
<i>Mean</i>		<b>4927.50</b>	<b>3969.67</b>	<b>0.97</b>	<b>1.00</b>	<b>2645.00</b>	<b>3969.67</b>	<b>6.25</b>	<b>12.85</b>
<i>RSD</i>		<b>1.32</b>	<b>0.35</b>	<b>0.59</b>	<b>1.00</b>	<b>1.72</b>	<b>0.35</b>	<b>0.80</b>	<b>0.43</b>

**Table (S2): System suitability tests of the proposed RP-LC method for the simultaneous determination of BREX and FLX.**

Parameters	FLX-A	BREX	FLX	Reference value **
N	2108	2489	3982	The higher the value, the more efficient the column is
T	1.12	0.99	1.05	≤2
R <sub>s</sub>	-	7.84	11.08	>2
α	-	3.72	3.08	≥1
K'	1.72	2.16	3.65	1-10

N: no of theoretical plates per column, T: tailing factor, α: Selectivity factor, K': Capacity factor, and R<sub>s</sub>: resolution.

\*\* U.S.P. Convention.2019. USP 46 NF41: United States Pharmacopeia [and] National Formulary, United States Pharmacopeial Convention.

**Table (S3): Eco-scale green assessment profile.**

Category	Method
<b>HPLC</b>	
1. Chemicals	Amount x hazard type x hazard amount
Ethanol	$1 \times 2 \times 2 = 4$
SLS	$1 \times 2 \times 3 = 6$ $= 4+6=10$
2. Occupational hazard	Hermetic sealing of the analytical process= 0
3. Energy (kWh/sample)	$\leq 1.5 = 1$
4. Waste	
a)Amount	$> 10 \text{ ml} = 5$
b)Waste treatment	Recycling =0
Total Penalty Points	16
Analytical eco-scale score= 100- total PPs	100-16= 84
Comment	excellent green analysis