Supplementary Information

Quantification of Solid State Impurity with Powder X-ray Diffraction using Laboratory Source

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Figure S24. HPLC overlay of 'unknown' samples (I, II and III) of BPO spiked API

1. Calculation of degree of crystallinity of BPO

Three sample preparations were analysed in a low background holder by an initial PXRD method (tables S1 and S2). Degree of crystallinity was calculated using EVA software suite (Bruker, DIFFRAC.EVA, User Manual, version 09.2017) as follows:

$$\%\ \text{Amorphous} = \frac{\text{Global area} - \text{Reduced area}}{\text{Global area}} \times 100$$

$$\%\ \text{Crystallinity} = 100 - \%\text{Amorphous}$$

Table S1. Degree of crystallinity calculation using EVA v.4.3.0

Degree of crystallinity of BPO				
BPO	**Preparation-1**	**Preparation-2**	**Preparation-3**	**Average Degree of Crystallinity (%)**
% Crystallinity	94.7	94.6	94.1	94.47

Table S2. Method parameters for initial PXRD method

Parameter	Parameter Values
Instrument Configuration	Bragg-Brentano geometry (e.g. Theta-theta)
Tube	Ceramic X-ray Cu anode (LFF) tube
Generator power	40 kV/40 mA
Detector	LYNXEYE
Soller slit	axial 2.5° primary/secondary
Divergent slit	0.3° primary
Active length of detector	2.949° 2θ
Diffracted beam anti scattering slit	8 mm (not automated)
Diffracted beam filter	Nickel $K\beta$ filter
Phi rotation (spinner)	On (15 rpm)
Scan Range	2 to 40° 2θ
Scan mode	Continuous
Step size	0.0388° 2θ
Time/Step	1.0 second

2. Preparation of BPO and API standard mixture

The batch of API used for preparing standard compositions of BPO spiked API samples contained 0.03% w/w (259 ppm) BPO as confirmed by HPLC analysis. Potency of the batch of BPO used for spiking studies was 90% w/w. The weight correction to the API and BPO samples were performed as follows:

$$\text{Weight of BPO in API taken in mg} = \frac{\text{Weight of API taken} \times \text{Percentage of BPO present in the API}}{100}$$

The content of BPO in standard mixtures was calculated using the corrected weights of API and BPO:

$$\text{Content of BPO } \left(\% \frac{\text{w}}{\text{w}}\right) = \frac{\text{Corrected BPO weight in mg}}{\text{Total weight}} \times \text{\% Potency}$$

Table S3. BPO and API stock standard mixture preparation

Weight of BPO (mg)	Weight of API (mg)	Weight of BPO in API (mg)	Corrected BPO weight (mg)	Corrected API weight (mg)	Total weight (mg)	Content of BPO (% w/w)
101.38	893.50	0.27	101.65	893.23	994.88	9.236

3. PXRD Method development

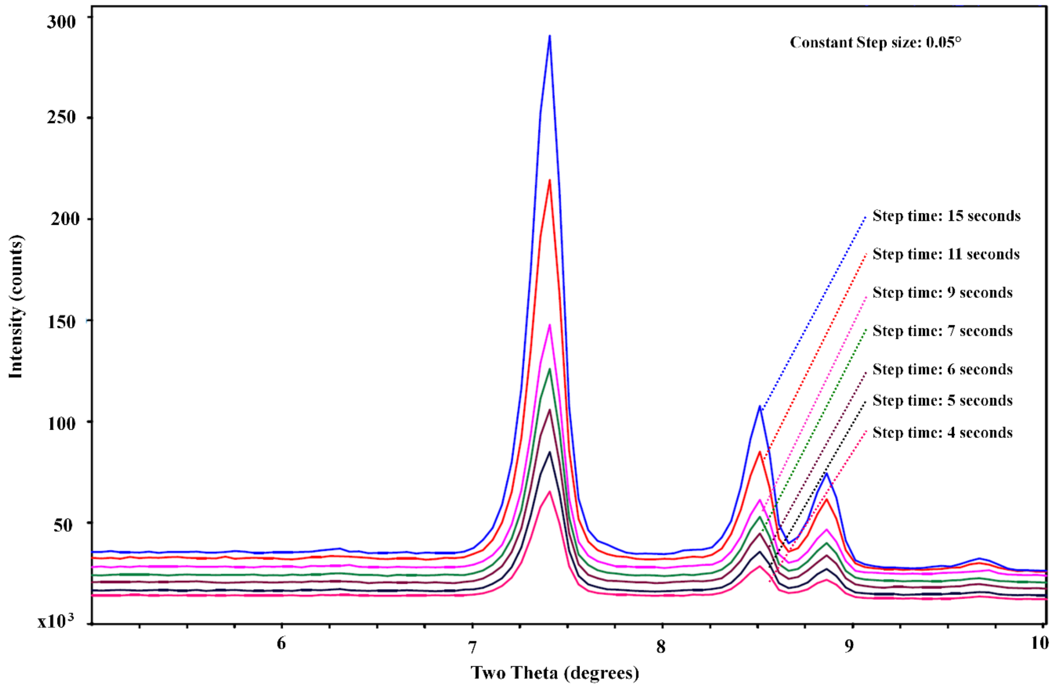

Figure S1. PXRD Overlay of ~0.45% w/w BPO in API standard mixture analysed with different step times (exposure time, seconds per step) with constant step size of 0.05°

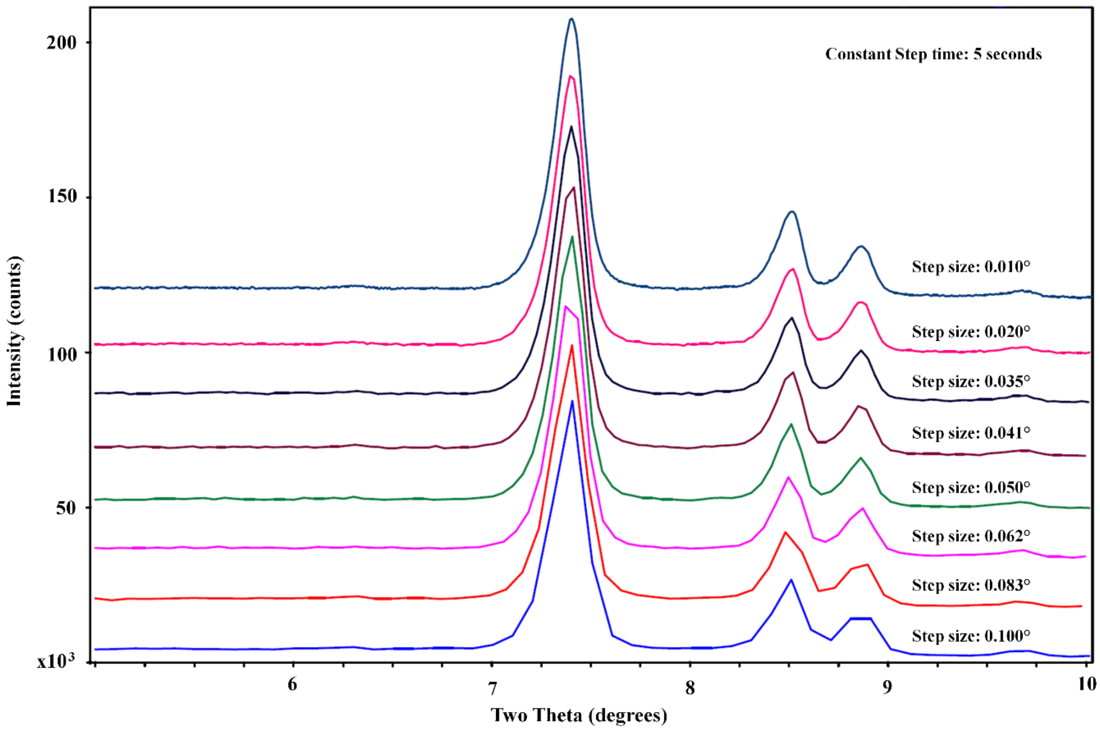

Figure S2. PXRD Overlay of ~0.45% w/w BPO in API standard mixture analysed with different step sizes with constant step time of 5 seconds per step

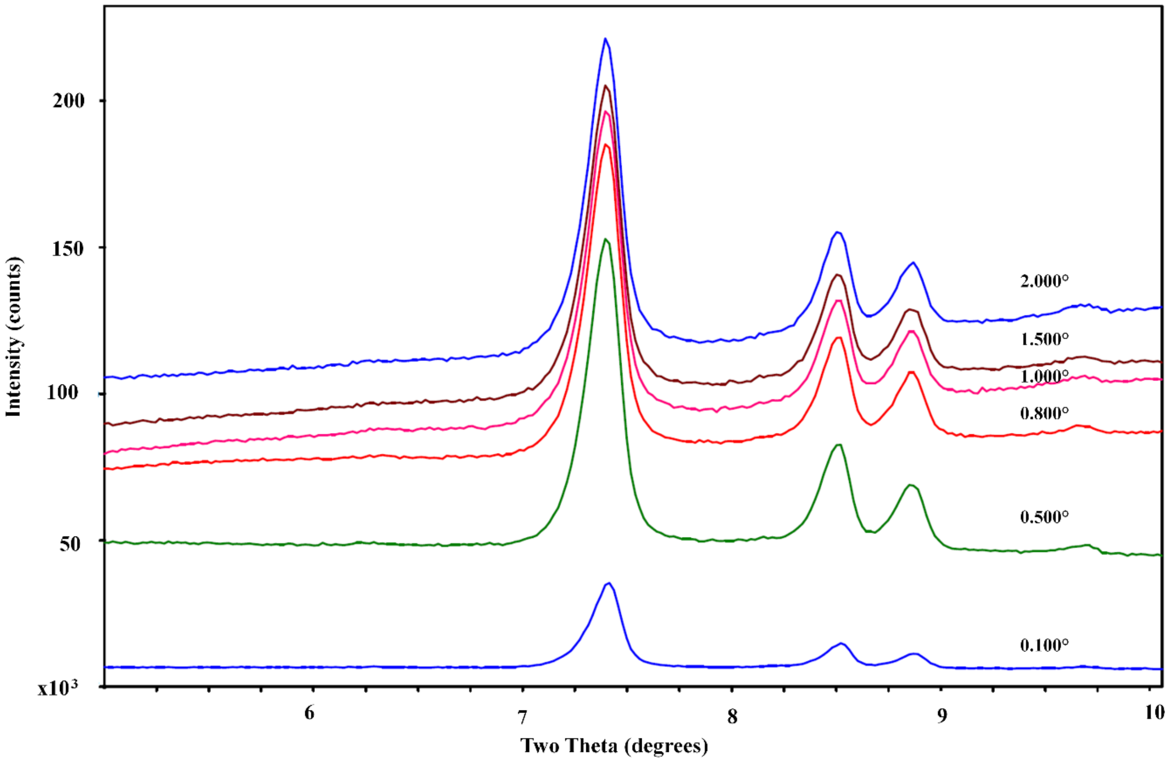

Figure S3. PXRD Overlay of ~0.90% w/w BPO in API standard mixture analysed with different divergence slit widths

4. Preparation of calibration curve

Table S4. Intensities (areas) of BPO and API peaks and calculation of area ratio for the preparation of calibration curve using different BPO and API standard mixtures

S. No.	Stock sample Weight in mg	Weight of API in mg	% w/w of BPO content	Area in counts at 7.38° 2θ	Area in counts at 8.86° 2θ	Area ratio	Average area ratio
1	41.11	411.68	0.866	1101029	150516	0.1203	0.1198
				1103768	152452	0.1214	
				1106388	149215	0.1188	
				1104540	153624	0.1221	
				1107447	146016	0.1165	
2	20.18	429.57	0.443	1113860	71710	0.0605	0.0592
				1116109	71374	0.0601	
				1118092	69409	0.0584	
				1111676	69726	0.0590	
				1116410	68629	0.0579	
3	15.18	435.03	0.340	1090902	48456	0.0425	0.0431
				1117212	50965	0.0436	
				1117067	51556	0.0441	
				1114184	48688	0.0419	
				1119202	50910	0.0435	
4	10.20	439.70	0.239	1083216	38575	0.0344	0.0325
				1080844	34991	0.0314	
				1082934	37228	0.0332	
				1085833	35828	0.0319	
				1085496	35174	0.0314	
5	5.02	445.12	0.133	1129288	17627	0.0154	0.0168
				1126209	20252	0.0177	
				1126390	20565	0.0179	
				1126454	18039	0.0158	
				1096074	19306	0.0173	
6	3.36	446.54	0.099	1049862	15184	0.0143	0.0132
				1058913	14908	0.0139	
				1052540	14603	0.0137	
				1058293	12582	0.0117	
				1055215	13067	0.0122	
7	1.84	448.67	0.068	999919	7834	0.0078	0.0070
				1001434	7330	0.0073	
				1006071	5992	0.0059	
				1006302	6775	0.0067	
				946956	7156	0.0075	
8	0.00	450.00	0.027	0	0	0.0000	0.0000
				0	0	0.0000	
				0	0	0.0000	
				0	0	0.0000	
				0	0	0.0000	

Slope	0.1405
STEYX	0.0015
Intercept	-0.0025
Correlation coefficient	0.9994
Limit of Detection	0.04
Limit of Quantitation	0.11
Residual sum of squares	1.17

Confidence interval of the regression line slope

Slope	0.1405
Std err in slope, Sb	0.0020
Degrees freedom	6
Confidence level	0.95
Student t	2.4469
Confidence interval	0.0048
Slope	0.1405 ± 0.0048
Lower limit	0.1357
Upper limit	0.1454

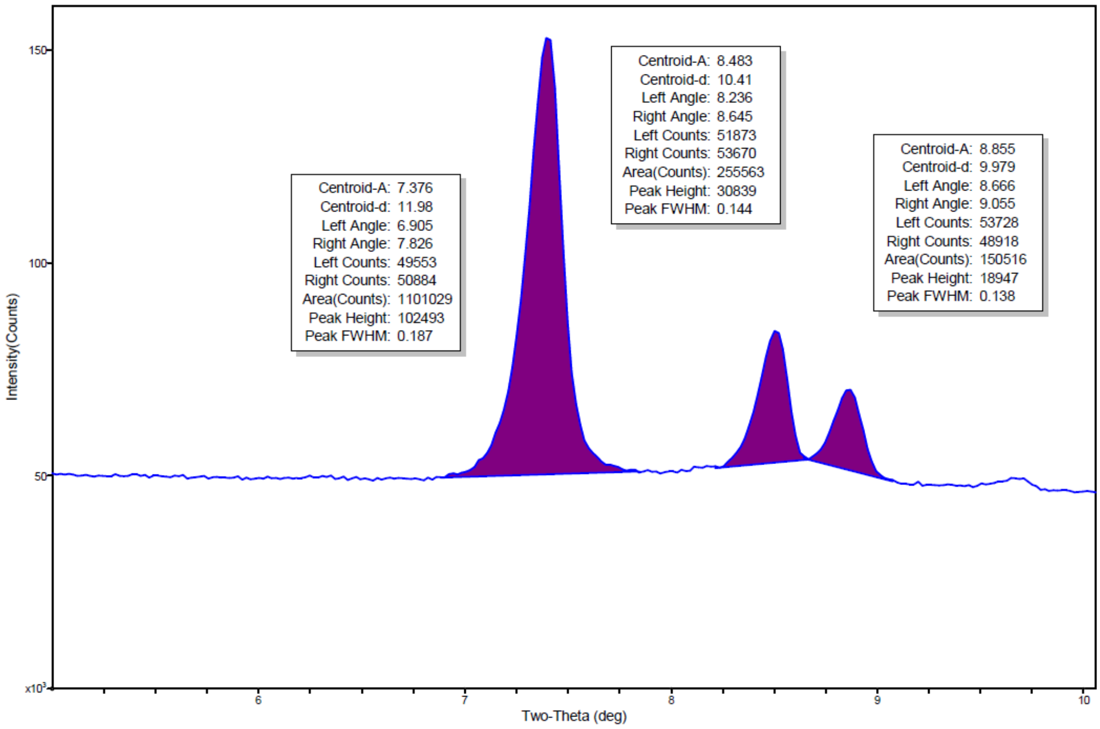

Figure S4. Measurement of BPO and API peak area of a 0.866% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

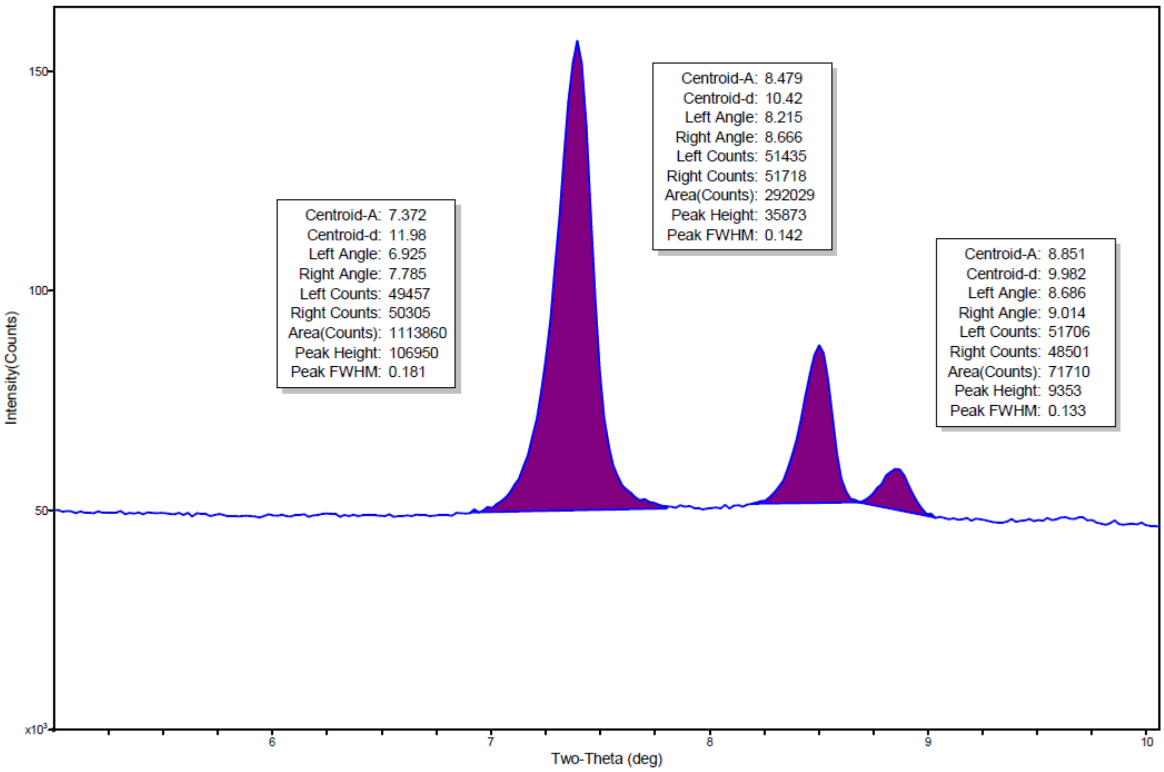

Figure S5. Measurement of BPO and API peak area of a 0.443% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

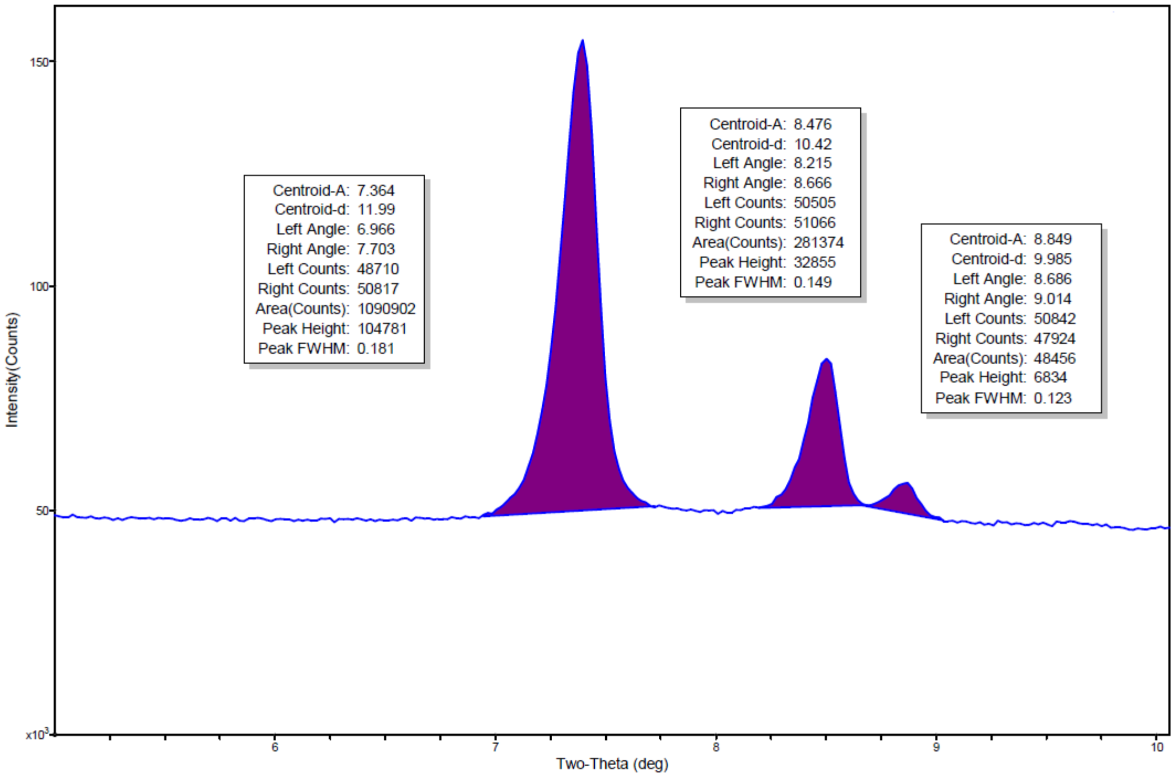

Figure S6. Measurement of BPO and API peak area of a 0.340% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

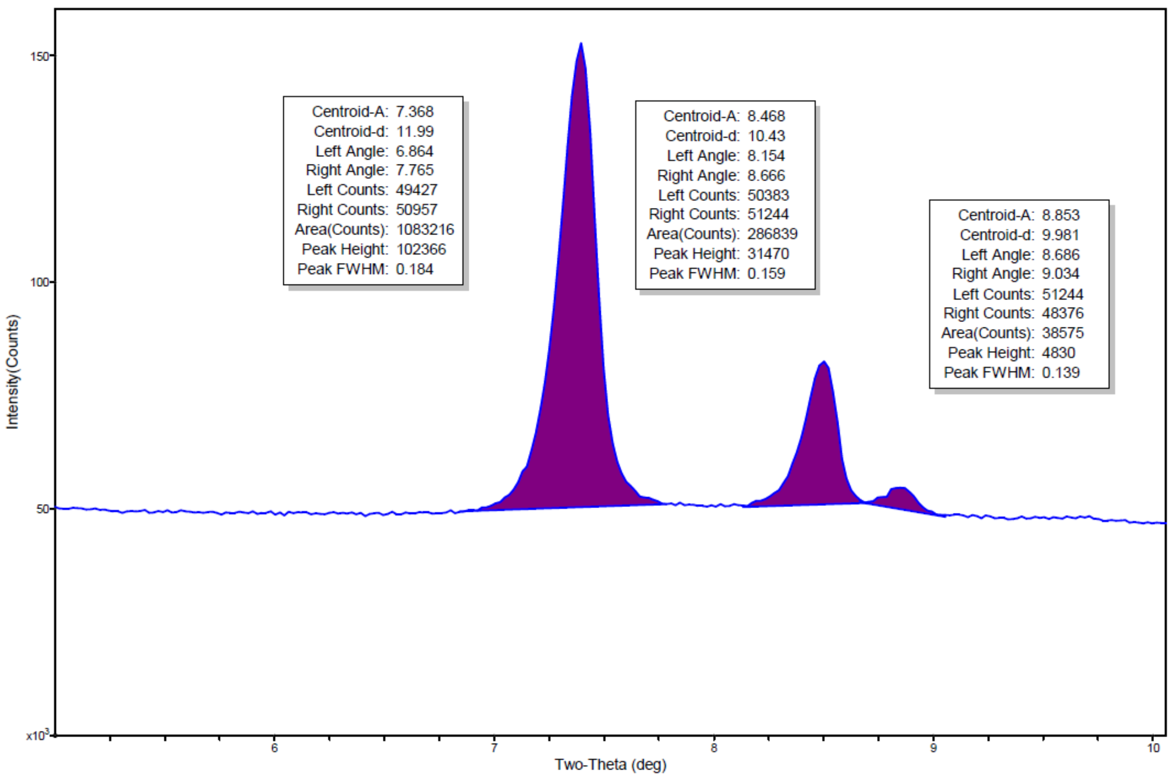

Figure S7. Measurement of BPO and API peak area of a 0.239% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

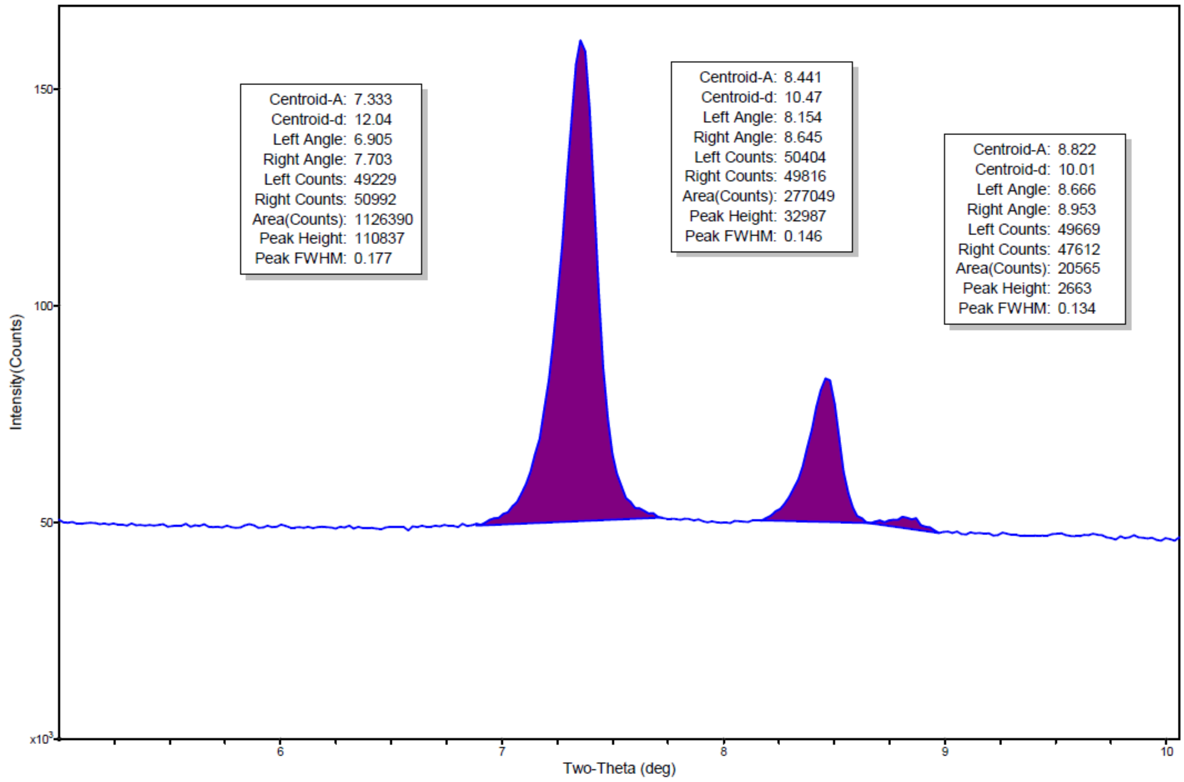

Figure S8. Measurement of BPO and API peak area of a 0.133% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

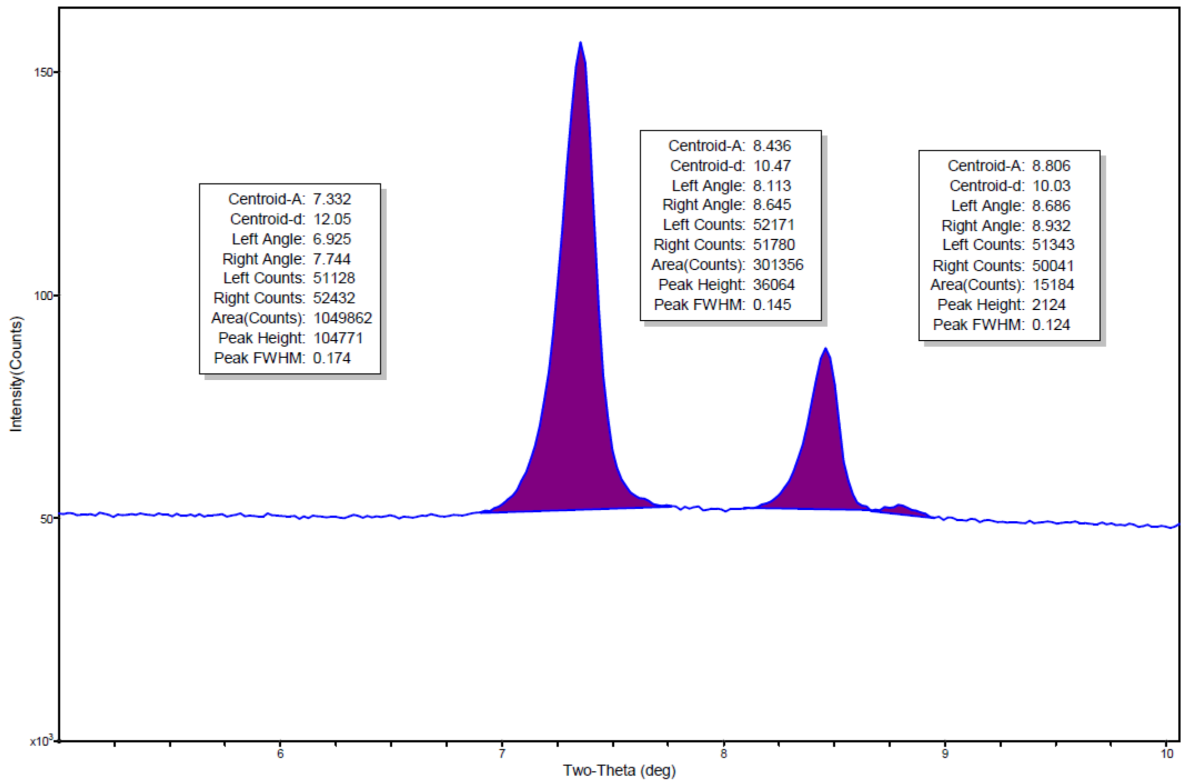

Figure S9. Measurement of BPO and API peak area of a 0.099% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

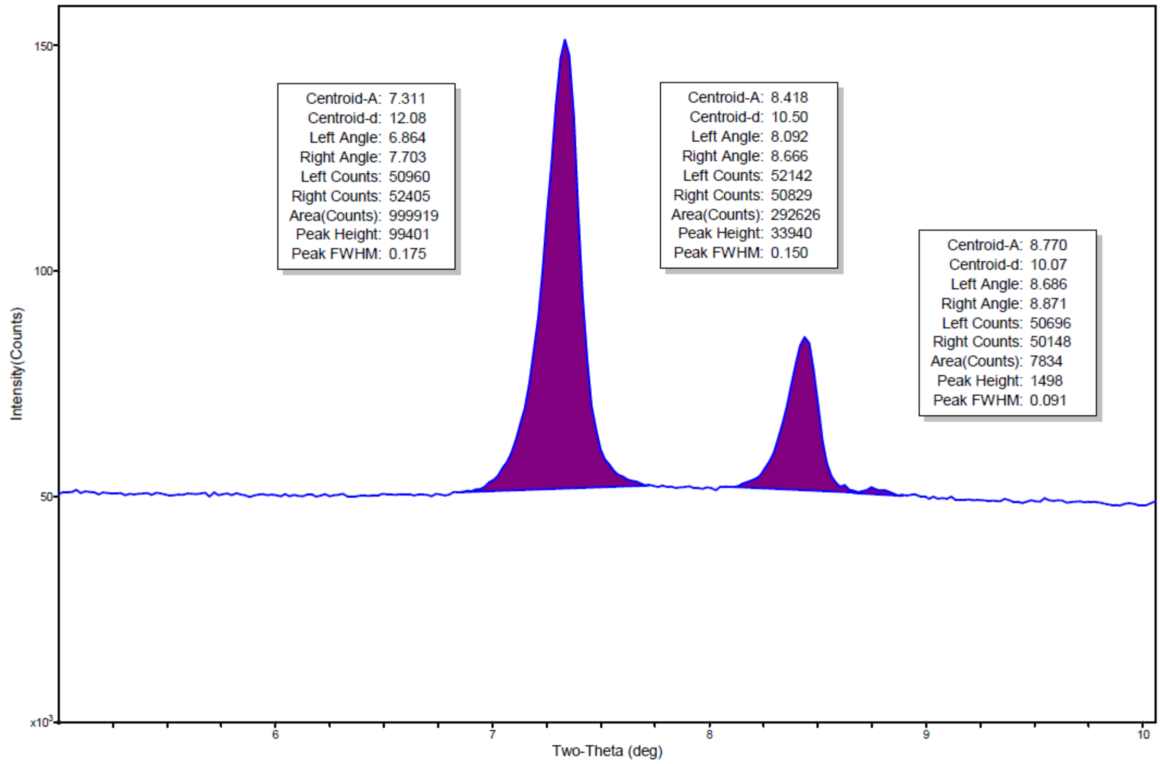

Figure S10. Measurement of BPO and API peak area of a 0.068% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

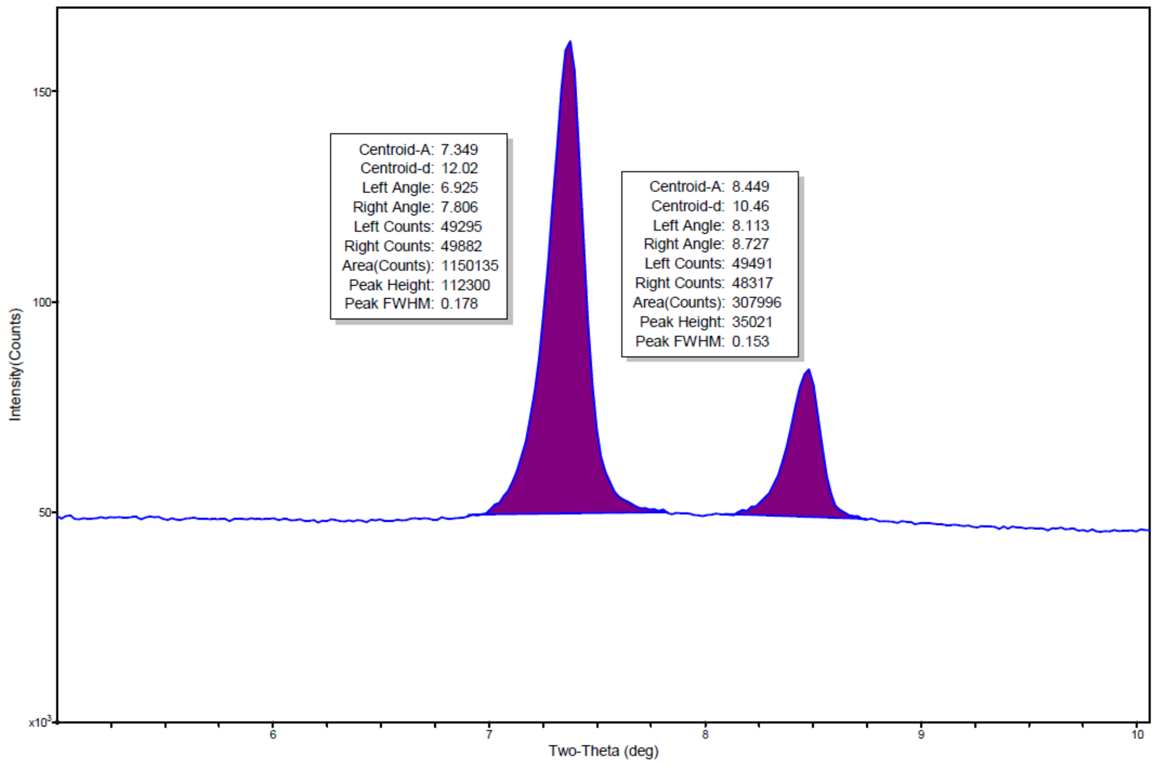

Figure S11. Measurement of API peak area of a 0.027% w/w BPO in API standard mixture using MDI JADE v. 9.5.0

4.1. Profile fitting (peak decomposition) analysis

A PXRD pattern from each of the spiking levels was analysed for profile fitting and peak decomposition. It was observed that all the peaks in the range 5-10° 2θ could be profile fitted satisfactorily for all the samples (Figures S12-S19). The resolution between the two closely spaced peaks namely, a peak at 8.48° 2θ of API and a peak at 8.86° 2θ of BPO was found to be greater than 1 in each case [1]. Area ratios obtained by intensity values from profile fitted peaks are comparable to that obtained from the manual integration and follow similar trend i.e. increase in the area ratio value with increase in BPO spiking concentration (Table S5).

Table S5. Comparison of the area ratios obtained by using intensity values (area under the curve) from profile fitted peaks (7.38° 2θ of API and 8.86° 2θ of BPO) and that from manual integration

S. No.	Sample (% w/w BPO in API)	Area ratio	
		Using intensity value from profile fitted peaks	Using intensity value from manual integration
1	0.866	0.1299	0.1198
2	0.443	0.0600	0.0592
3	0.340	0.0395	0.0431
4	0.239	0.0318	0.0325
5	0.133	0.0161	0.0168
6	0.099	0.0112	0.0132
7	0.068	0.0072	0.0070
8	0.027	0	0

[1] Resolution with next adjacent peak (R_s) was calculated as:

$$R_s = \frac{X_{c2} - X_{c1}}{0.5\,(w_2 + w_1)}$$

Where X_{c1} and X_{c2} are peak centers, and w_1 and w_2 are constructed base widths.

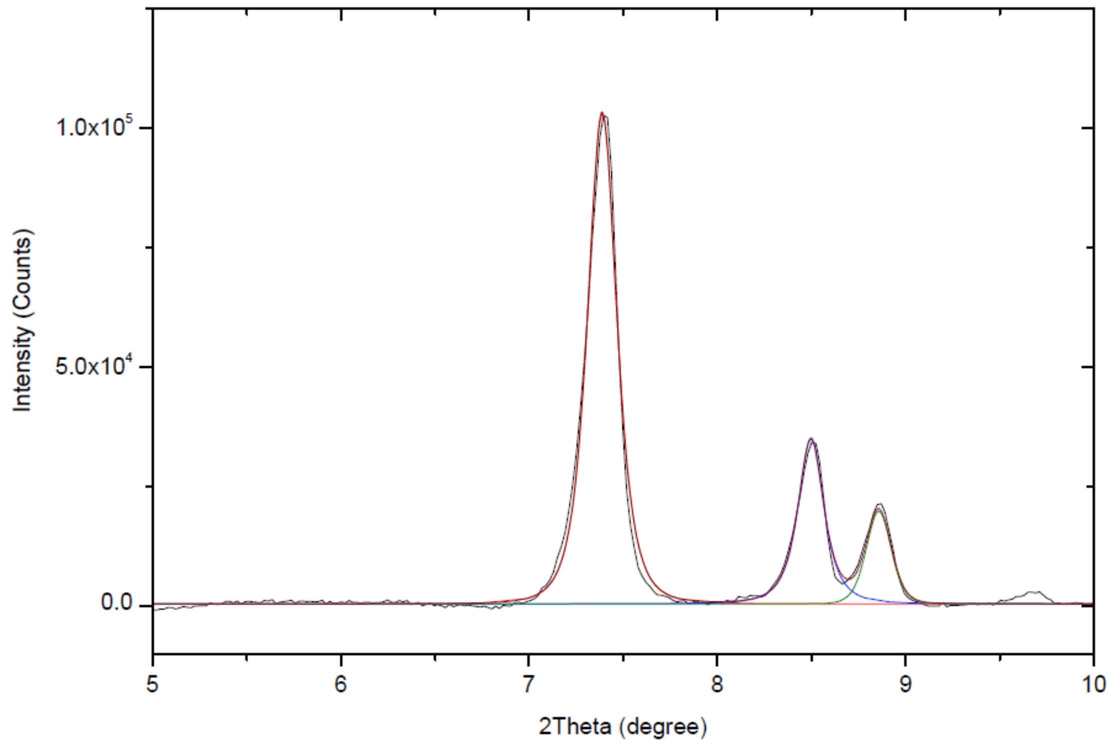

Figure S12. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.866% w/w BPO in API standard mixture sample

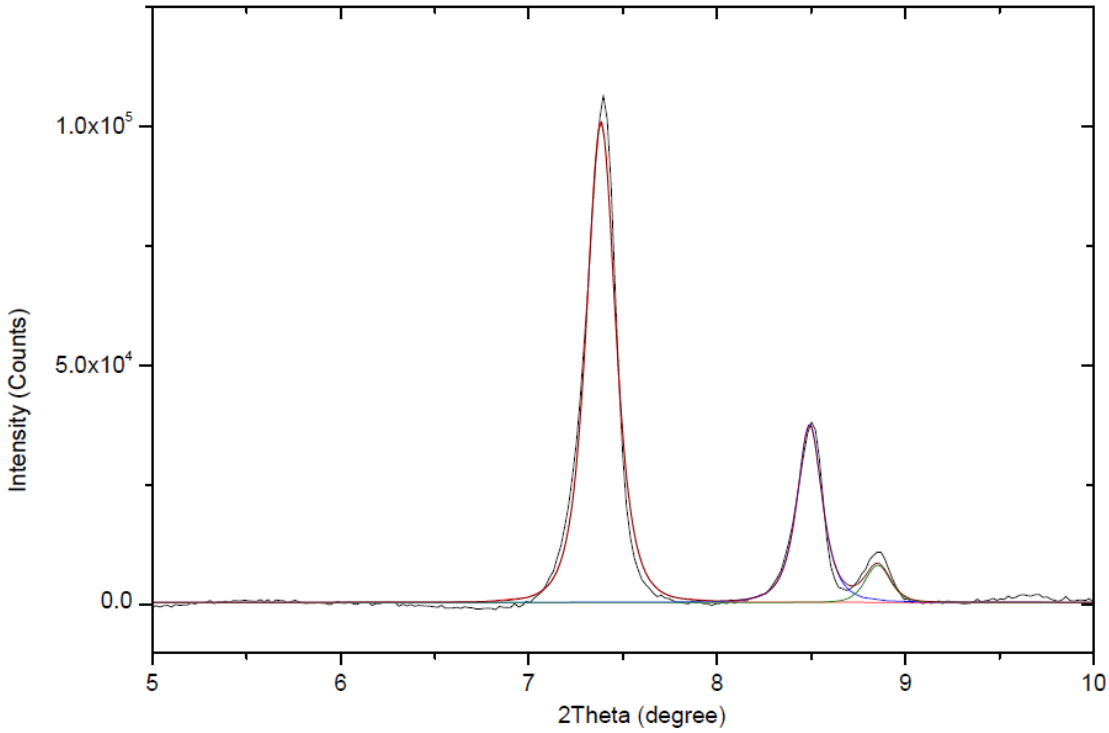

Figure S13. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.443% w/w BPO in API standard mixture sample

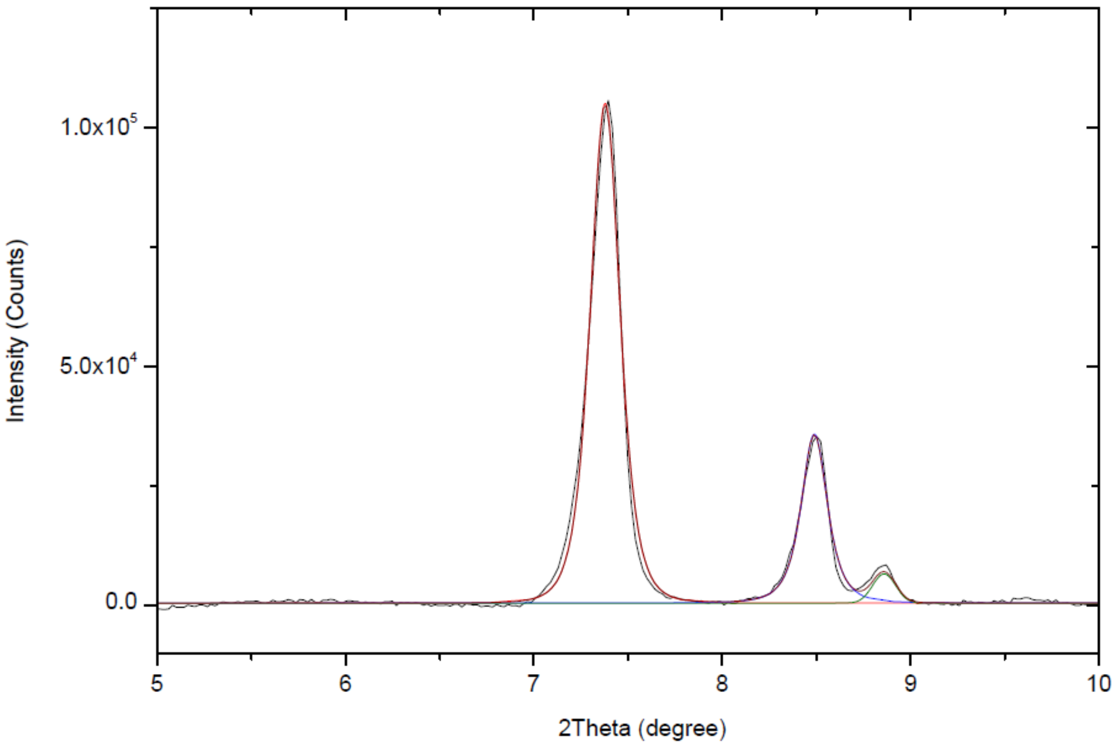

Figure S14. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.340% w/w BPO in API standard mixture sample

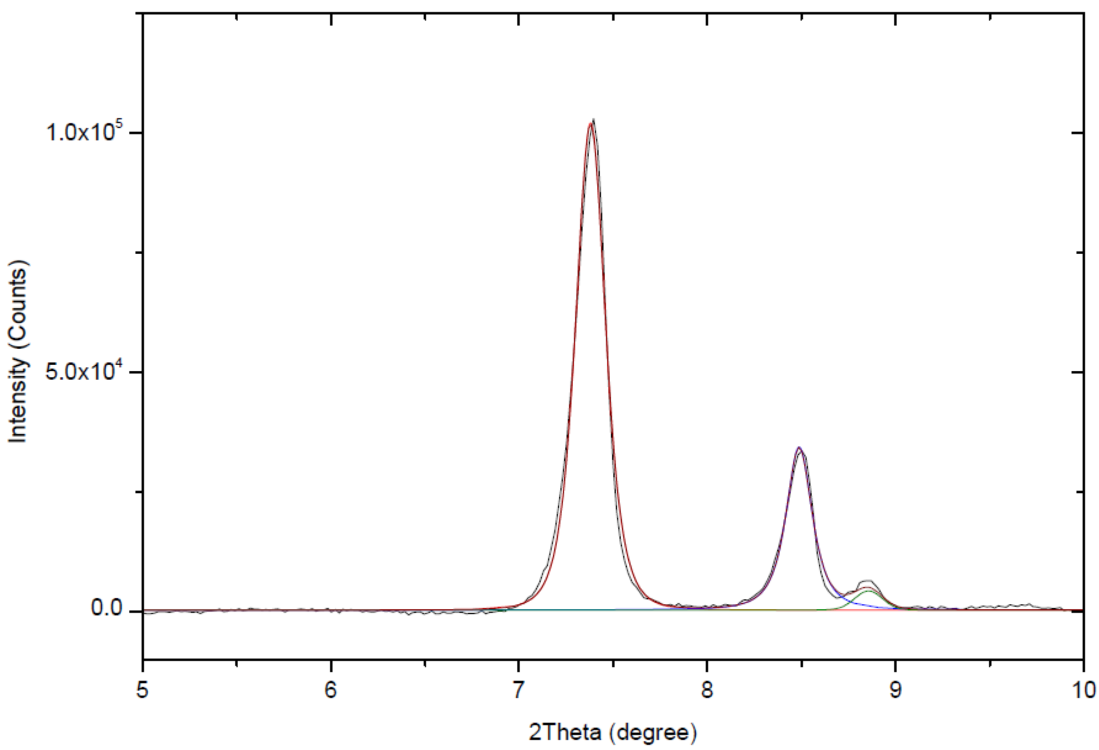

Figure S15. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.239% w/w BPO in API standard mixture sample

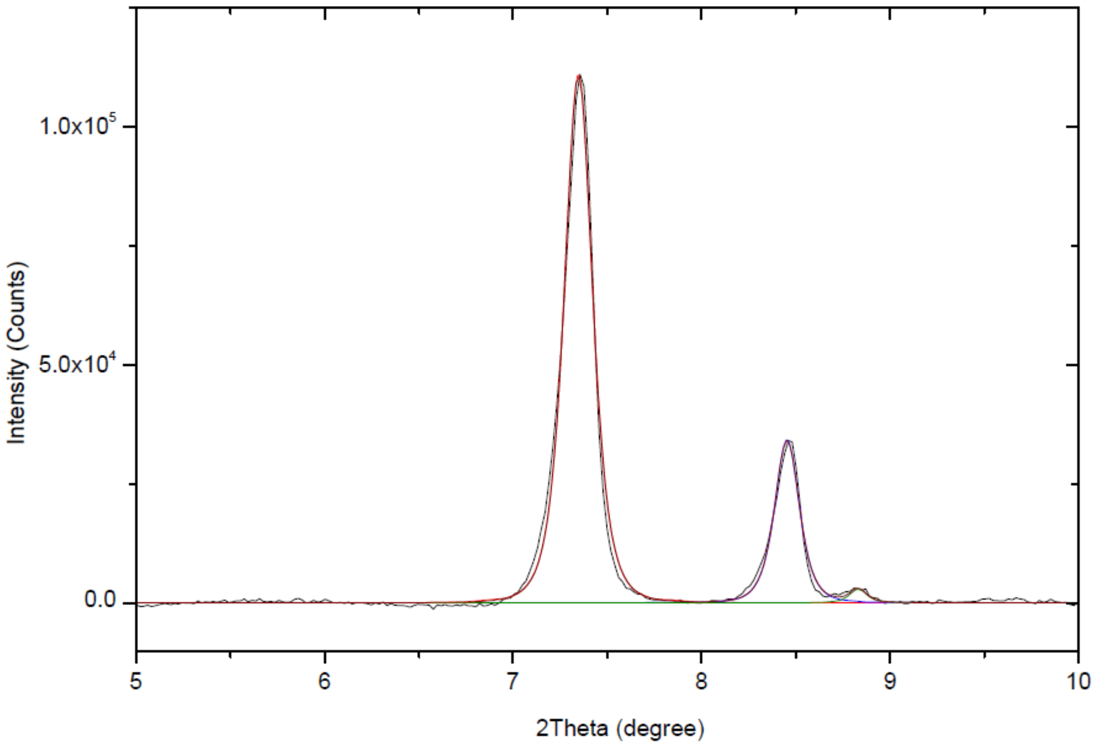

Figure S16. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.133% w/w BPO in API standard mixture sample

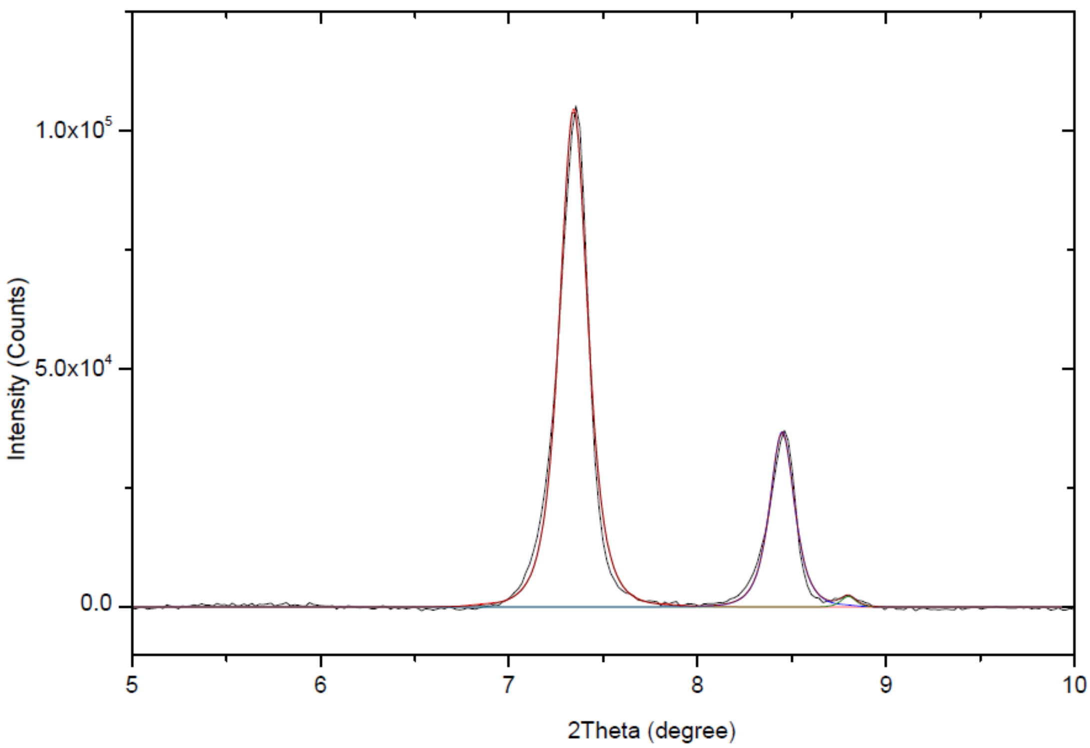

Figure S17. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.099% w/w BPO in API standard mixture sample

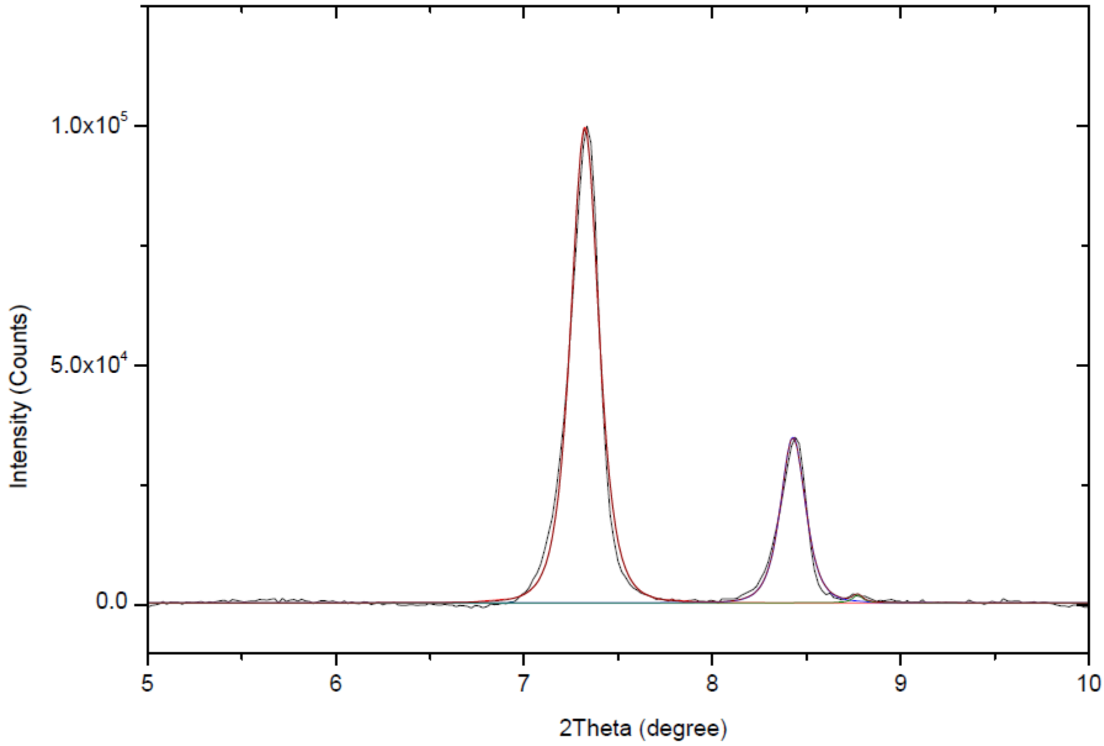

Figure S18. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.068% w/w BPO in API standard mixture sample

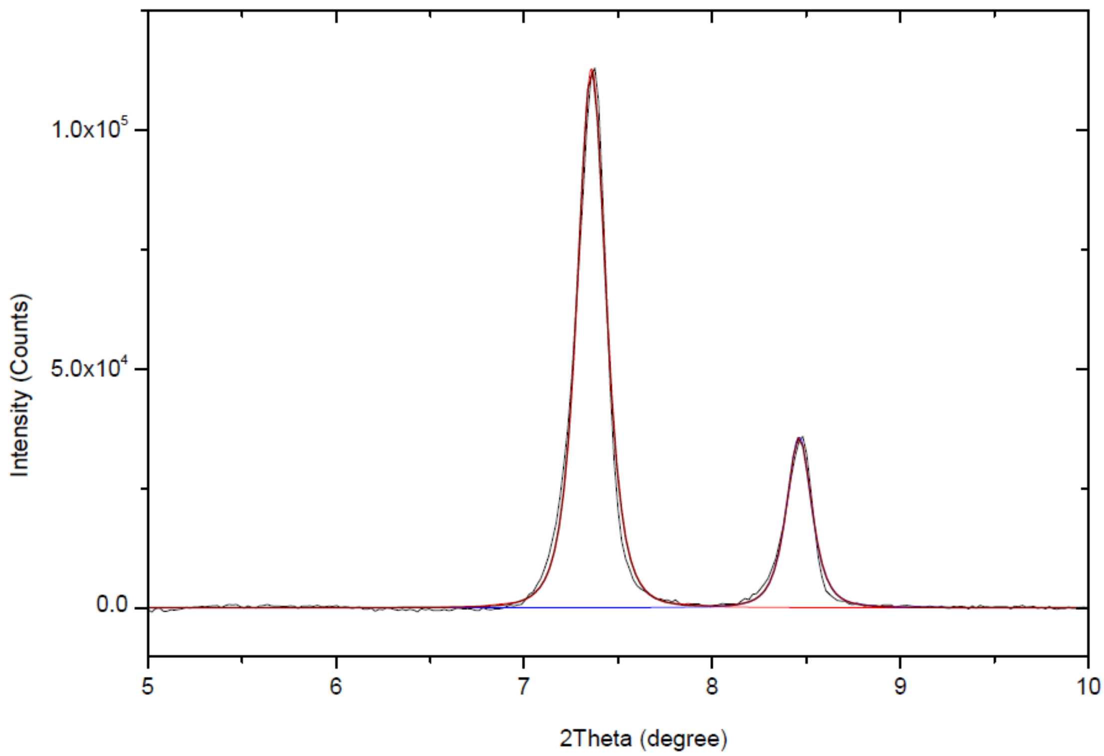

Figure S19. Profile fitting (peak decomposition) analysis for a PXRD pattern of 0.027% w/w BPO in API standard mixture sample

5. PXRD method validation

Table S6. Range studies at 0.90% and 0.10% w/w BPO in API standard mixtures

S.No.	% w/w of BPO content	Area in counts at 7.38° 2θ	Area in counts at 8.86° 2θ	Area ratio
Range at 0.90% w/w BPO in API standard mixture				
1	0.866	1101029	150516	0.1203
2		1103768	152452	0.1214
3		1106388	149215	0.1188
4		1104540	153624	0.1221
5		1107447	146016	0.1165
6		1101337	144264	0.1158
	Mean			**0.119**
	st.DEV			**0.0026**
	%RSD			**2.18**
Range at 0.10% w/w BPO in API standard mixture				
S.No.	**% w/w of BPO content**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**
1	0.099	1049862	15184	0.0143
2		1058913	14908	0.0139
3		1052540	14603	0.0137
4		1058293	12582	0.0117
5		1055215	13067	0.0122
6		1045791	13837	0.0131
	Mean			**0.013**
	st.DEV			**0.0010**
	%RSD			**7.69**

Table S7. Method precision determined using 0.45%, 0.35% and 0.25% w/w BPO in API standard mixtures

S.No.	% w/w of BPO content	Area in counts at 7.38° 2θ	Area in counts at 8.86° 2θ	Area ratio	Mean	st.DEV	%RSD
1	0.443	1113860	71710	0.0605	0.060	0.001	**1.83**
2		1116109	71374	0.0601			
3		1118092	69409	0.0584			
4	0.340	1090902	48456	0.0425	0.043	0.001	**1.86**
5		1117212	50965	0.0436			
6		1117067	51556	0.0441			
7	0.239	1083216	38575	0.0344	0.033	0.002	**4.55**
8		1080844	34991	0.0314			
9		1082934	37228	0.0332			

Table S8. Precision determination for standard mixture corresponding to LOQ (0.133% w/w BPO in API) and for next available lower level (0.099% w/w BPO in API)

0.133% w/w BPO in API binary mixture Precision				
S.No.	**% w/w of BPO content**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**
1	0.133	1129288	17627	0.0154
2		1126209	20252	0.0177
3		1126390	20565	0.0179
4		1129758	19574	0.0170
5		1126454	18039	0.0158
6		1096074	19306	0.0173
	Mean			**0.017**
	st.DEV			**0.0010**
	%RSD			**5.88**
Limit of Quantification level (0.099% w/w BPO in API binary mixture) precision				
S.No.	**% w/w of BPO content**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**
1	0.099	1049862	15184	0.0143
2		1058913	14908	0.0139
3		1052540	14603	0.0137
4		1058293	12582	0.0117
5		1055215	13067	0.0122
6		1045791	13837	0.0131
	Mean			**0.013**
	st.DEV			**0.0010**
	%RSD			**7.69**

Table S9. Precision determination for standard mixture corresponding to LOD (0.068% w/w BPO in API)

S.No.	**% w/w of BPO content**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**
1	0.068	999919	7834	0.0078
2		1001434	7330	0.0073
3		1006071	5992	0.0059
4		1006820	7996	0.0079
5		1006302	6775	0.0067
6		946956	7156	0.0075
	Mean			**0.007**
	st.DEV			**0.0007**
	%RSD			**10.00**

Table S10. Accuracy determination using 0.45%, 0.15% and 0.10% w/w BPO in API standard mixtures with calculated slope (0.1405) and intercept (-0.0025) values from the calibration curve

S.No.	% w/w of BPO content	Area in counts at 7.38° 2θ	Area in counts at 8.86° 2θ	Area ratio	Average Area ratio	Calculated %w/w of BPO	% Recovery
1	0.443	1113860	71710	0.0605	0.060	0.460	99.59
		1116109	71374	0.0601			
		1118092	69409	0.0584			
2	0.133	1126209	20252	0.0177	0.018	0.149	91.15
		1126390	20565	0.0179			
		1129758	19574	0.0170			
3	0.099	1049862	15184	0.0143	0.014	0.119	84.30
		1058913	14908	0.0139			
		1052540	14603	0.0137			

Table S11. Estimation of assay errors such as Instrument Repeatability with 0.90% w/w BPO in API standard mixture and Intra/Inter Day Repeatability with 0.45% w/w BPO in API standard mixture

Instrument Repeatability				
S.No.	**% w/w of BPO content**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**
1	0.866	1101029	150516	0.1203
2		1103768	152452	0.1214
3		1106388	149215	0.1188
4		1104540	153624	0.1221
5		1107447	146016	0.1165
6		1101029	150516	0.1203
7		1103768	152452	0.1214
8		1106388	149215	0.1188
9		1104540	153624	0.1221
10		1107447	146016	0.1165
			Mean	**0.1198**
			st.DEV	**0.0021**
			%RSD	**1.75**

Intra- and Inter-day Repeatability					
S.No.	**Day**	**% w/w of BPO content**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**
1	Day-1	0.443	1113860	71710	0.0605
2	Day-1		1116109	71374	0.0601
3	Day-1		1118092	69409	0.0584
4	Day-1		1111676	69726	0.0590
5	Day-1		1116410	68629	0.0579
6	Day-2	0.443	1120000	73930	0.0619
7	Day-2		1112534	69494	0.0588
8	Day-2		1105735	72832	0.0618
9	Day-2		1110533	71867	0.0608
10	Day-2		1113639	70299	0.0594
				Mean	**0.0599**
				st.DEV	**0.0014**
				%RSD	**2.31**

6. HPLC method details and validation

Table S12. HPLC method of analysis for BPO

Column	Waters Xbridge C18, 100 mm x 4.6 mm i.d., 3.5 μm particle size		
Column Part No.	186003033		
Column Temperature	25°C		
Sample Temperature	25°C		
Detector Wavelength	205 nm		
Pump Configuration	Gradient		
Flow Rate	1.0 mL/minute		
Injection Volume	10.0µL		
Run Time	20 minutes		
Mobile Phase A	0.05% v/v TFA MilliQ Water: Acetonitrile/ 90:10 v/v		
Mobile Phase B	0.05% v/v TFA MilliQ Water: Acetonitrile/ 10:90 v/v		
Needle Wash	Acetonitrile: Water (1:1 v/v)		
Diluent	DMSO		
Gradient Programme	Time (min)	% of Mobile phase A	% of Mobile phase B
	0	40	60
	5	0	100
	15	0	100
	17	40	60
	20	40	60
Retention Time of BPO		~ 9.1 minutes	

Standards Preparation:

Standard Stock Solution: Weighed about 110 mg of BPO (corrected for potency) in 100 mL volumetric flask, dissolved and diluted to volume with acetonitrile

Standard solution-1 (1% w/v level): Pipetted out 10 mL of standard stock solution in 100 mL volumetric flask, diluted to volume with diluent

Standard solution-2 (0.1% w/v level): Pipetted out 10 mL of standard solution-1 in 100 mL volumetric flask, diluted to volume with diluent

Standard solution-3 (0.05% w/v level): Pipetted out 5 mL of standard solution-1 in 100 mL volumetric flask, diluted to volume with diluent

Standard solution-4 (0.01% w/v level): Pipetted out 1 mL of standard solution-1 in 100 mL volumetric flask, diluted to volume with diluent

Sample concentration: Weighed about 100 mg of sample in 10 mL, dissolved and diluted to volume with diluent

Table S13. HPLC data for preparation of linearity curve using standard solutions with different levels of BPO

Linearity			
	Weight of BPO (mg)		110.08
	Potency of BPO (%)		90
Level	**Concentration**	**Area**	**Average Area**
0.01%	0.001101	7695 7621	7658
0.05%	0.005504	41111 42153	41632
0.10%	0.011008	83912 84361	84137
0.30%	0.033024	267631 265710	266671
0.50%	0.055040	401946 400614	401280
0.80%	0.088064	630628 630705	630667
1.0%	0.110080	861943 861119	861531
	Correlation Coefficient		**0.9979**

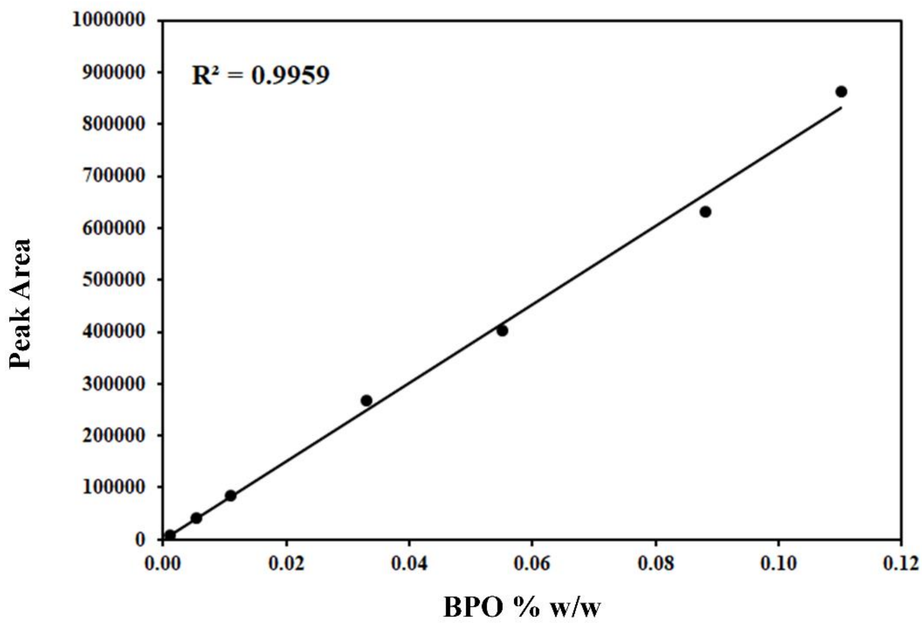

Figure S20. HPLC linearity curve plotted using peak area of BPO against concentration of BPO in the standard solutions

Table S14. HPLC Injection Precision for 0.10% and 1.00% w/w BPO standard solutions

0.10% Level		1.00% Level	
Injections	**Area**	**Injections**	**Area**
1	727390	1	7294956
2	728938	2	7308388
3	727270	3	7289689
4	728671	4	7293163
5	725630	5	7324544
Average	**727580**	**Average**	**7302148**
SD	**1320**	**SD**	**14389**
% RSD	**0.18**	**% RSD**	**0.20**

Table S15. HPLC % Recovery at 0.01%, 0.10% and 1.00% w/w BPO spiked in API

Sample Wt (mg)		Sample Wt (mg)		Sample Wt (mg)		Sample Wt (mg)	
102.52 (As Such)		102.38 (Spiked)		101.44 (Spiked)		100.2 (Spiked)	
Area	**Ave.Area**	**Area**	**Ave.Area**	**Area**	**Ave.Area**	**Area**	**Ave.Area**
163914	164558	234037	234116	877626	878049	7382585	7385649
165201		234194		878472		7388712	
% Recovery		93.1		98.2		99.5	
		0.01% Level		**0.10% Level**		**1.0% Level**	

Table S15. HPLC Precision at LOQ level 0.01% w/w BPO standard solution

Injection	Area
1	7695
2	7621
3	7623
4	7664
5	7739
Average	**7668**
Standard Deviation	**50.0**
% RSD	**0.65**

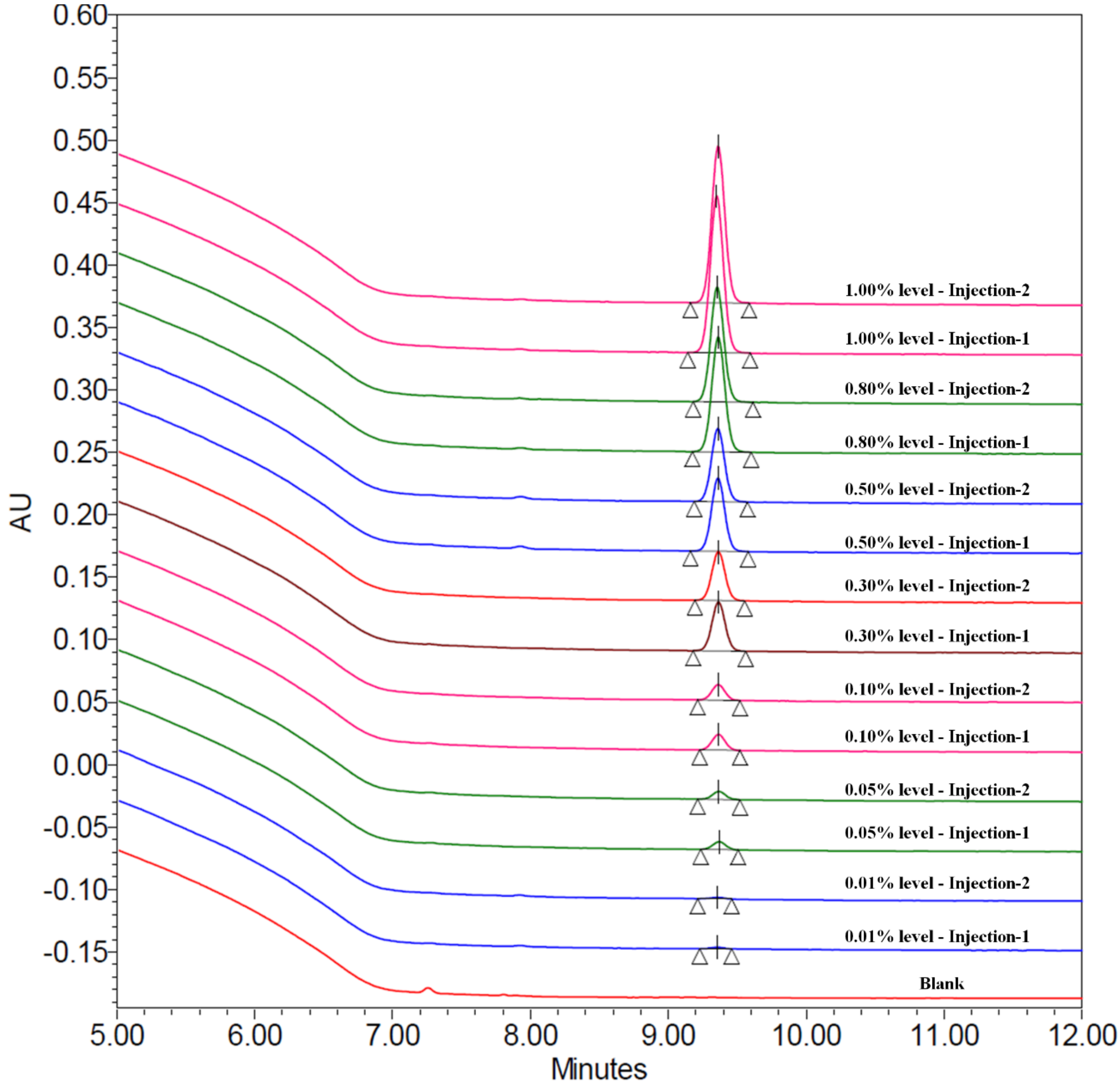

Figure S21. HPLC overlay of different levels of BPO analysed for Linearity

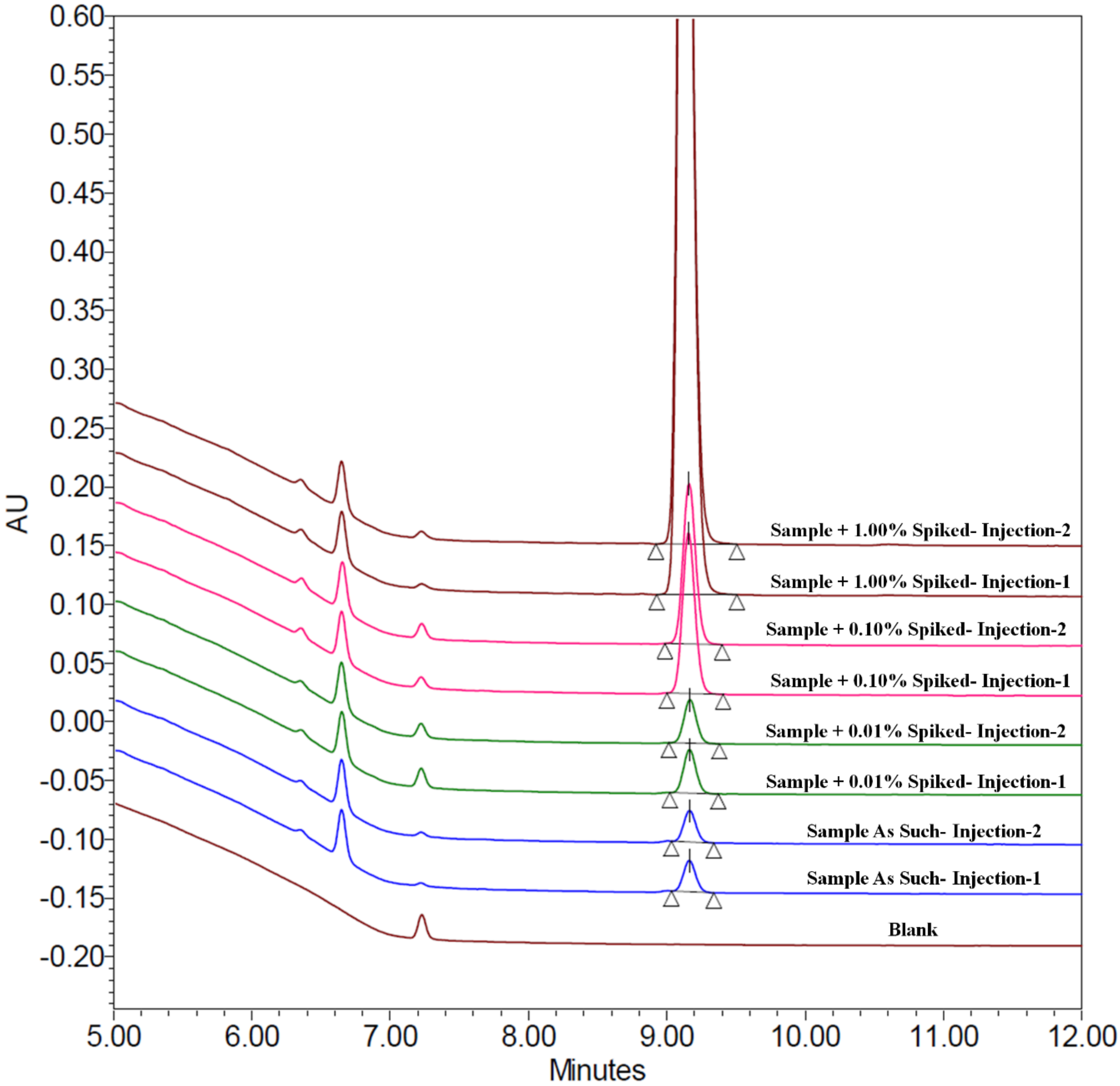

Figure S22. HPLC Overlay of different levels (0.01%, 0.10% and 1.00%w/w) of BPO spiked in API analysed for HPLC method Recovery

7. Comparison of PXRD and HPLC results for 'unknown' samples

Samples with three different levels of BPO spiking in API were prepared for verification of results obtained from PXRD calibration curve and its comparison with results obtained by HPLC technique.

Table S17. Concentration of BPO in API determined using PXRD and HPLC

PXRD						
S.No.	**Sample**	**Area in counts at 7.38° 2θ**	**Area in counts at 8.86° 2θ**	**Area ratio**	**Average Area ratio**	**BPO content (% w/w in API)**
1	I	610637	59155	0.0883	0.093	0.680
		603940	61642	0.0926		
		615290	66514	0.0976		
2	II	654480	32007	0.0466	0.048	0.359
		657285	33204	0.0481		
		655223	33150	0.0482		
3	III	693432	8302	0.0118	0.011	0.096
		698202	7960	0.0113		
		699662	7543	0.0107		

HPLC				
S.No.	**% w/w of BPO in API**	**Area of Brettphos Oxide**	**Average Area**	**BPO content (% w/w in API)**
1	I	4607899	4600305.5	0.695
		4592712		
2	II	2485301	2483538	0.381
		2481775		
3	III	716821	716096	0.108
		715371		

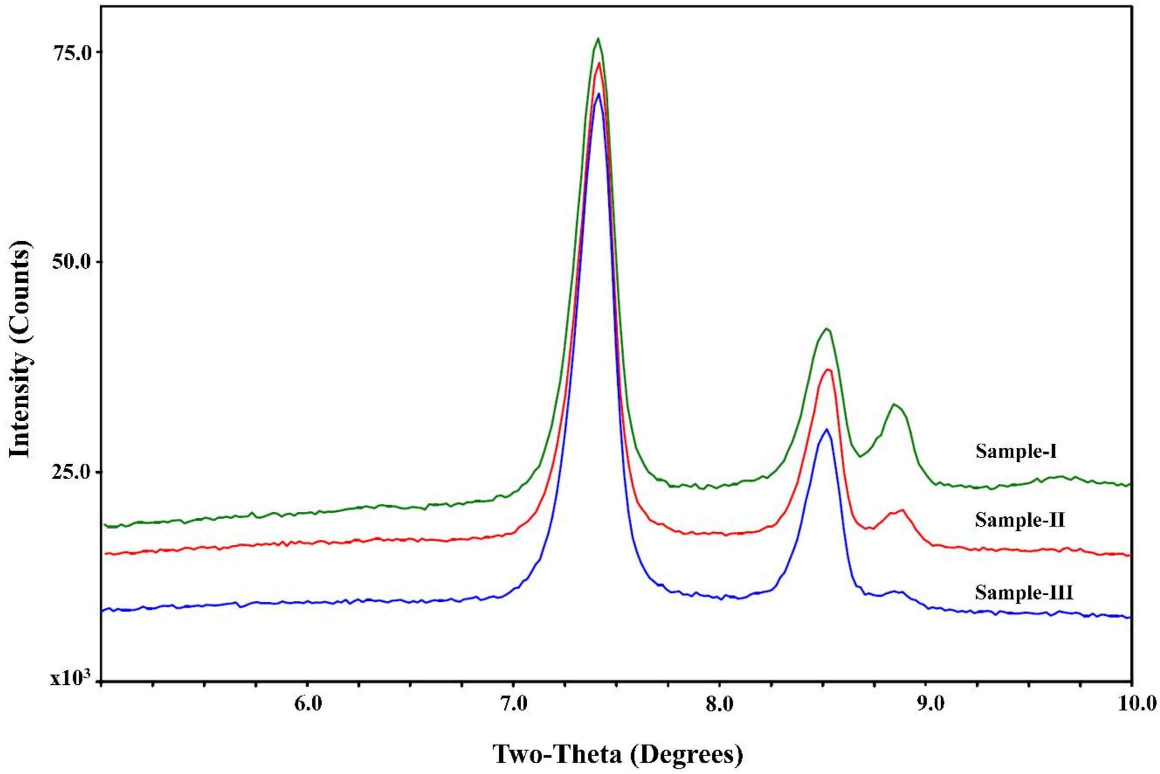

Figure S23. PXRD overlay of 'unknown' samples (I, II and III) of BPO spiked API

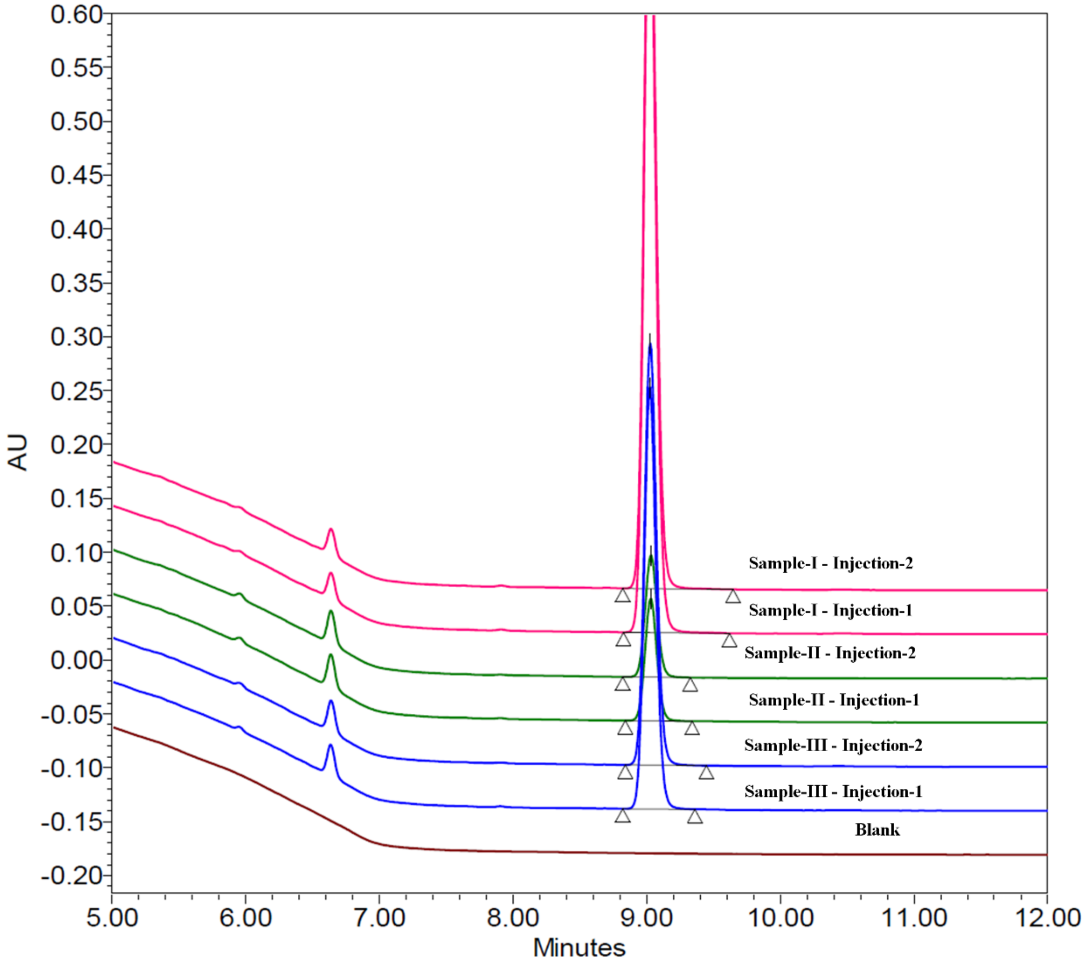

Figure S24. HPLC overlay of 'unknown' samples (I, II and III) of BPO spiked API