

THE ANALYSIS OF CLOBETASOL AND CLOBETASOL-17-PROPIONATE IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Project Scope

Analytical platform: Liquid chromatography tandem mass spectrometry

Matrix: Human Plasma

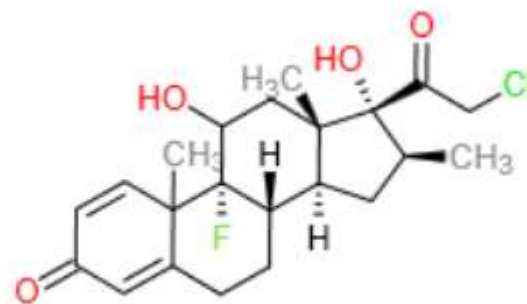
Anticoagulant: Lithium Heparin

Range: 500 fold

Lower limit of quantification: 5 pg/mL

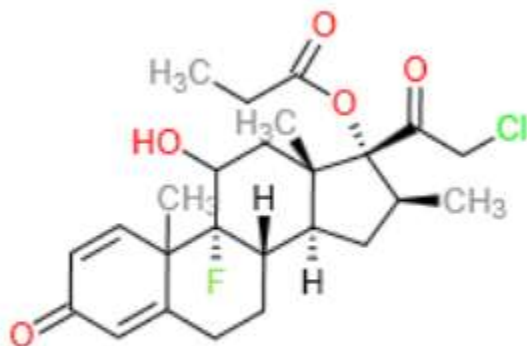
Literature methods: 40 pg/mL

Clobetasol



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Clobetasol-17-Propionate

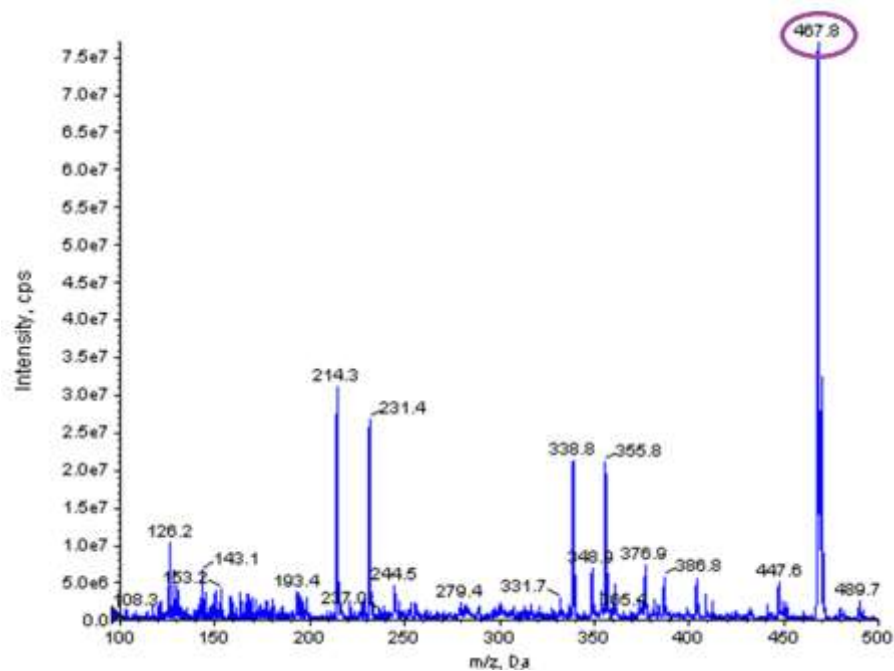


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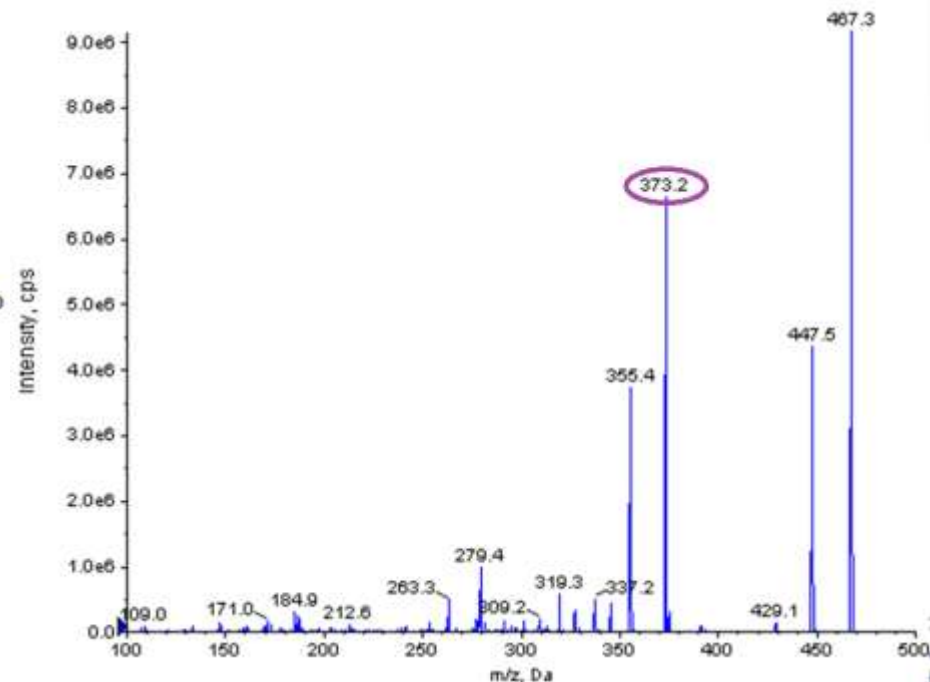
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Mass Spectrometer Optimisation – Clobetasol-17-Propionate

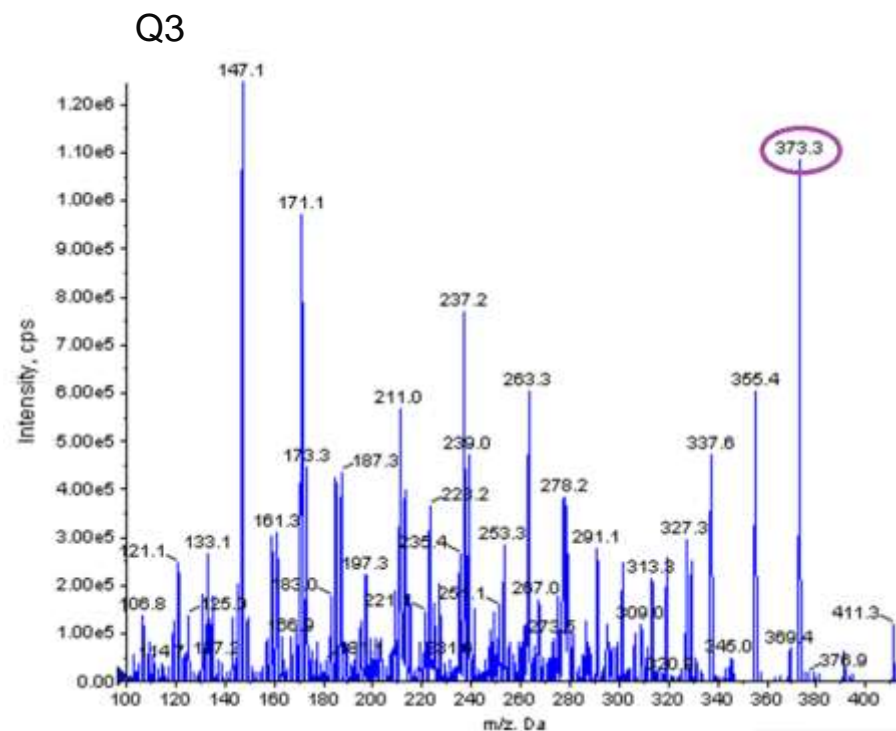
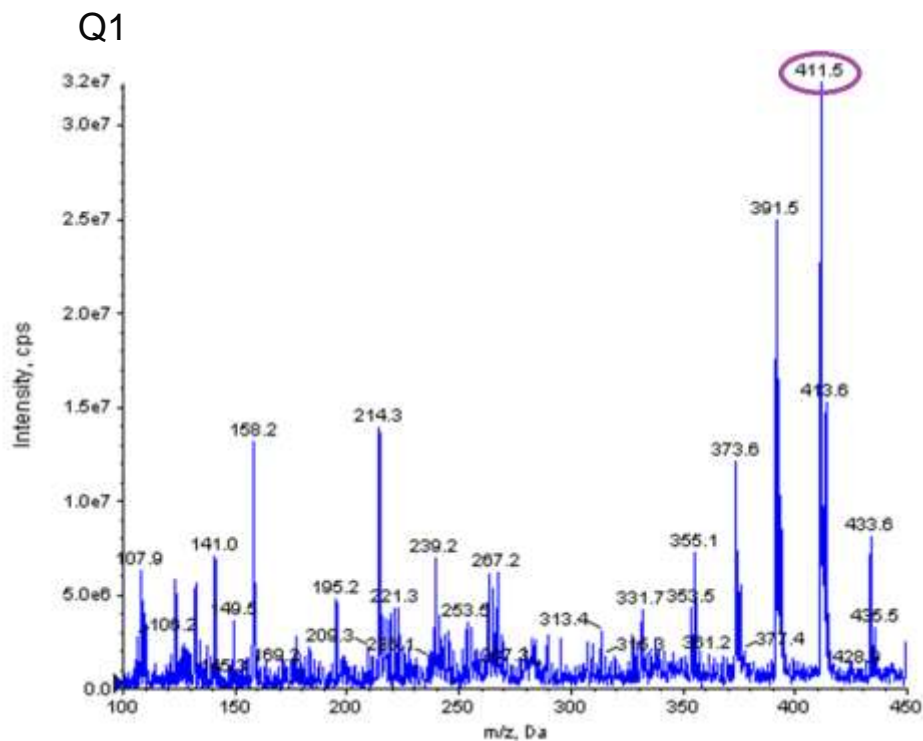
Q1



Q3



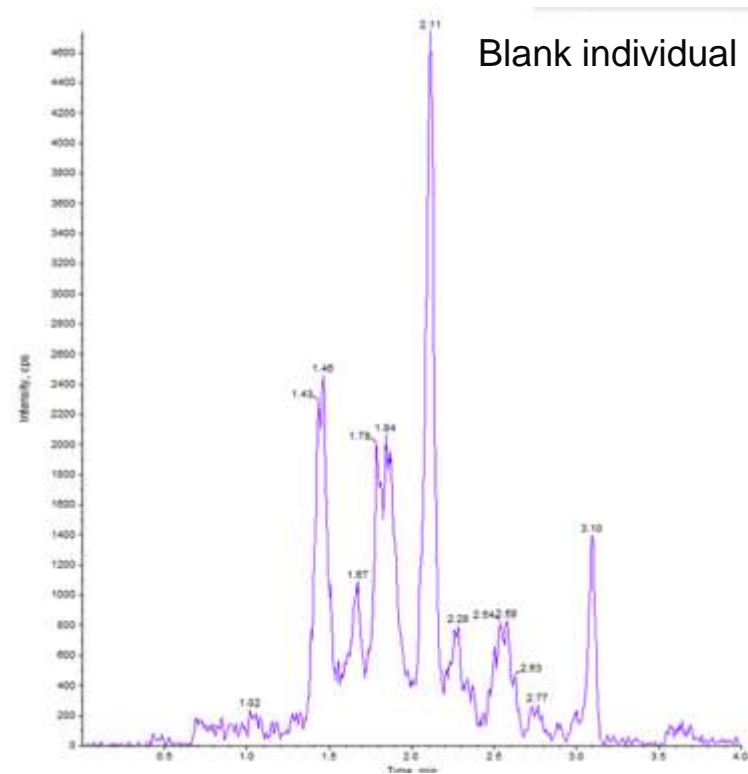
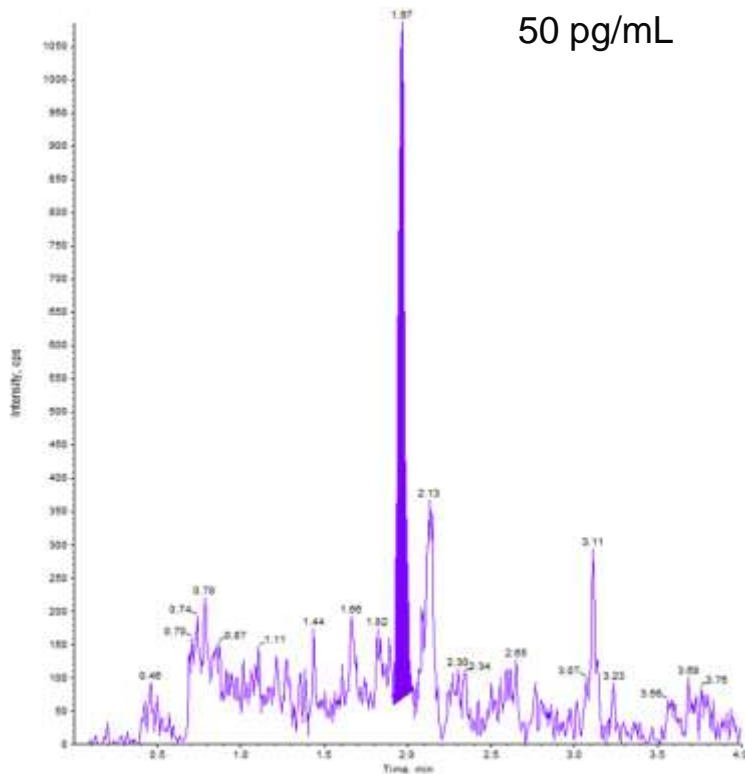
Mass Spectrometer Optimisation - Clobetasol



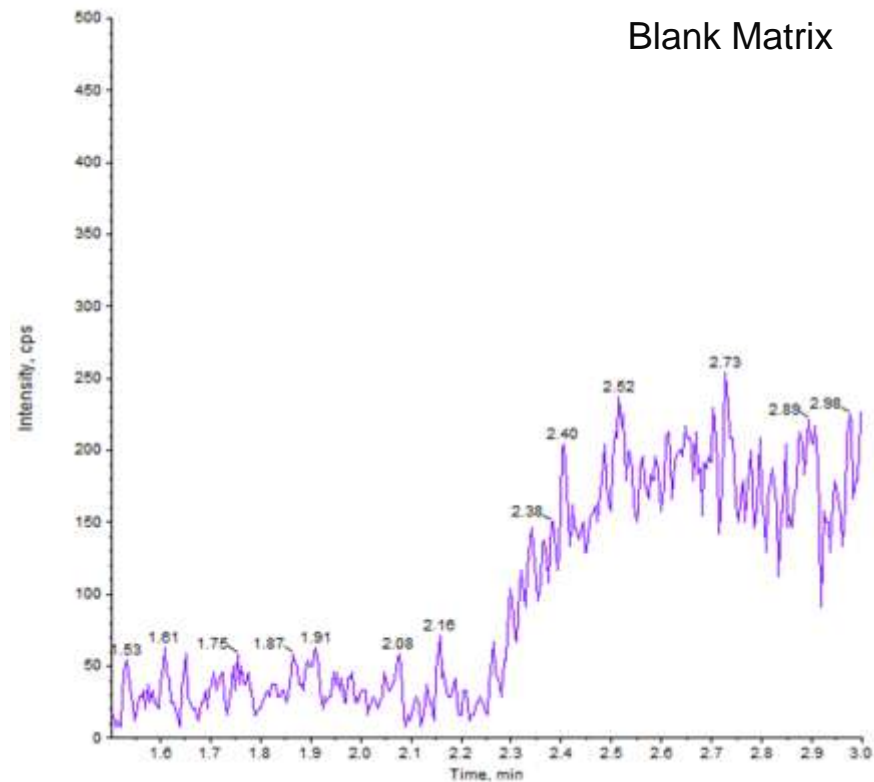
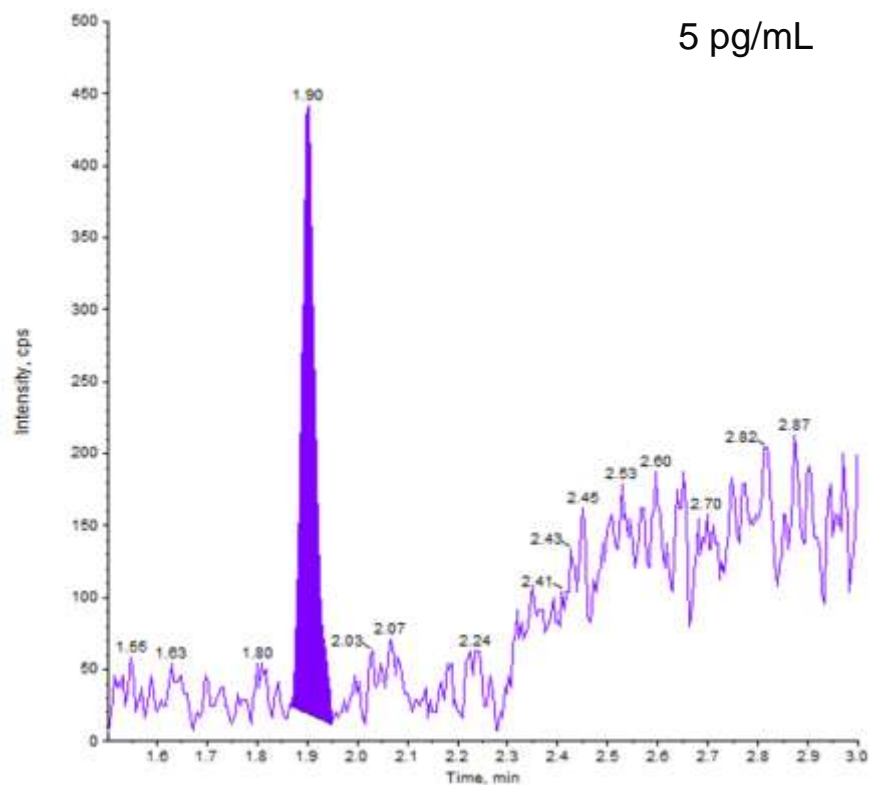
Protein Precipitation with Acetonitrile and Online Solid Phase Extraction

Clobetasol:

- ▶ Interfering peaks
- ▶ Suppression

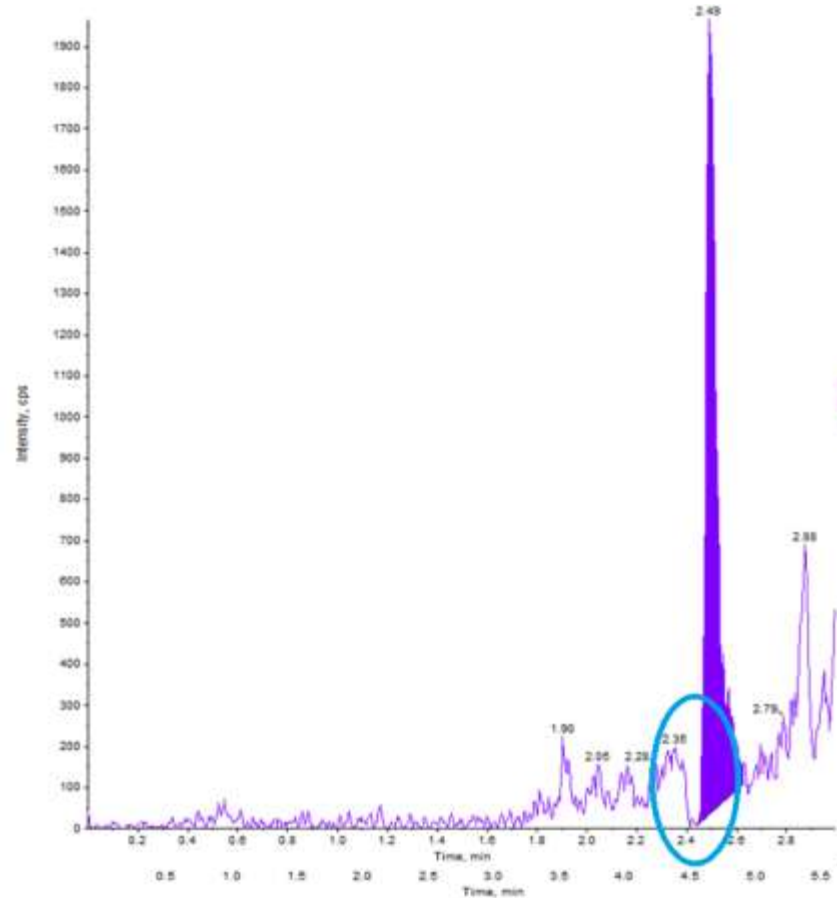
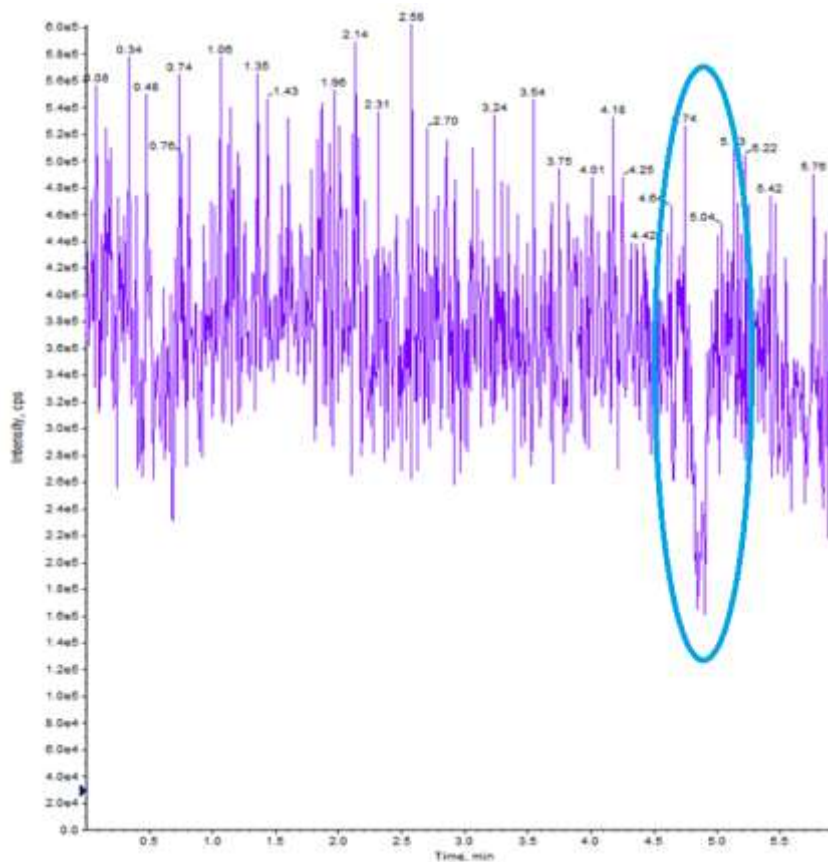


Supported Liquid Extraction: Clobetasol-17-Propionate



Supported Liquid Extraction: Clobetasol

- ▶ Interfering peaks
- ▶ Suppression, varying across different lots of matrix



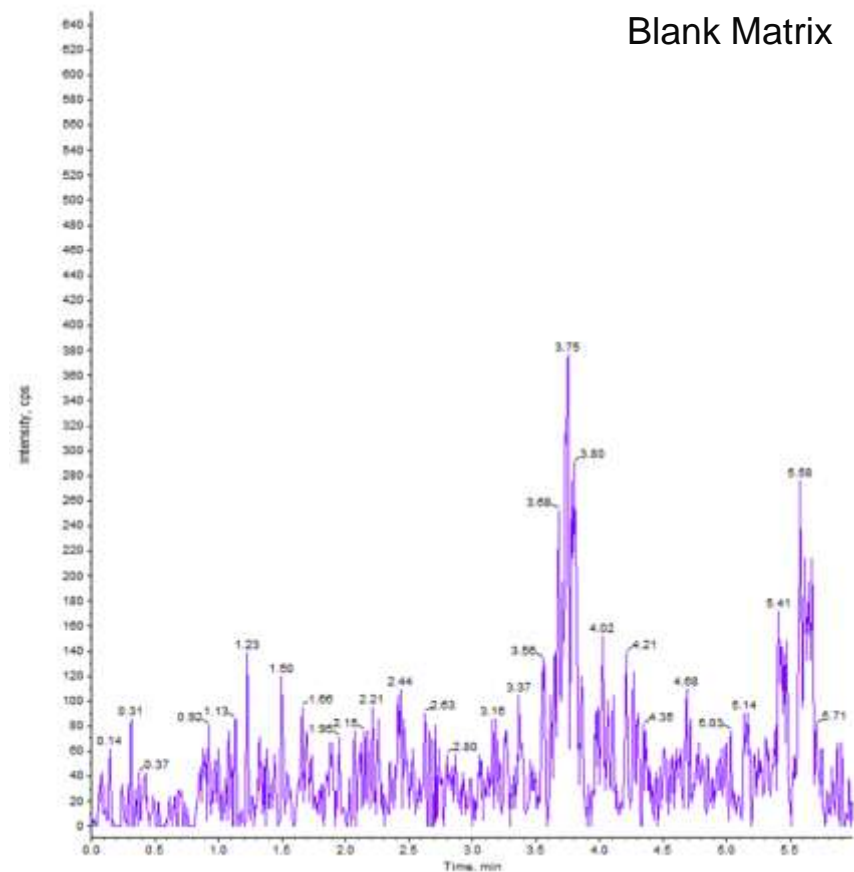
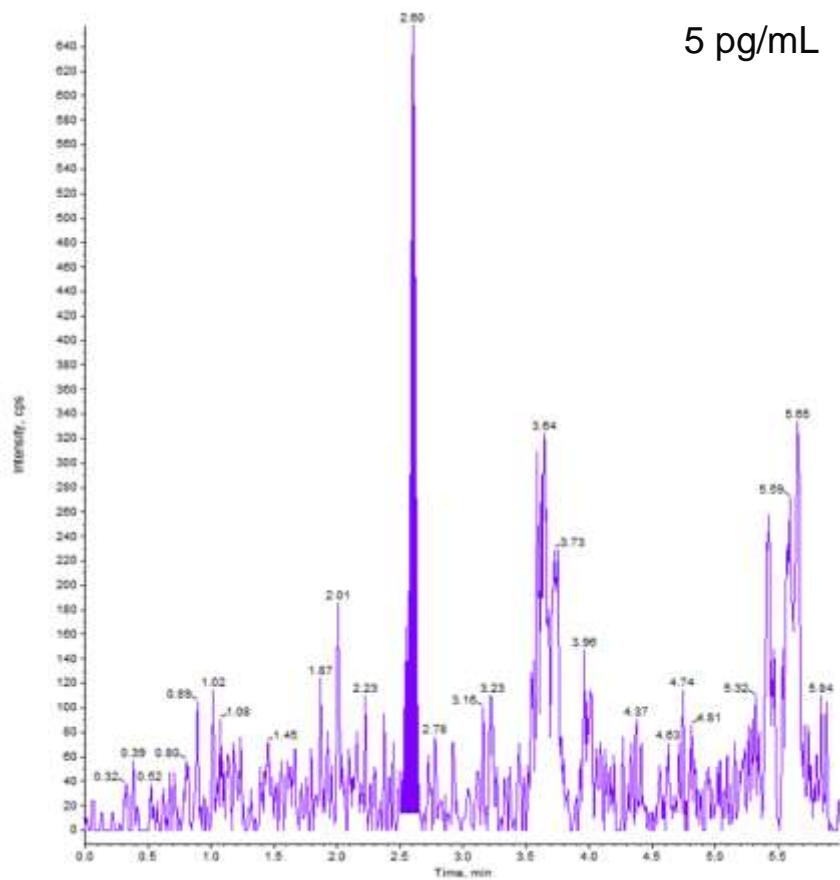
Supported Liquid Extraction: Clobetasol-17-Propionate

Expected concentration (pg/mL)	Sample name	Mean (pg/mL)	Accuracy %	Number of values
5	Cal 1	4.98	99.7	2
10	Cal 2	10.4	104	2
20	Cal 3	18.5	92.5	2
50	Cal 4	50.1	100	2
200	Cal 5	205	102	2
800	Cal 6	810	101	2
2000	Cal 7	2010	100	2
4500	Cal 8	4410	98	2
5000	Cal 9	5060	101	2

Passes acceptance criteria

Expected concentration (pg/mL)	Sample name	Mean (pg/mL)	% RSD	Accuracy	Number of values
5	LLOQ QC	4.66	6.86	93.2	6
15	Low QC	14.2	5.75	94.7	6
15	Low QC Individual 1	14.9	2.49	99.4	3
15	Low QC Individual 2	14	2.73	93.1	3
15	Low QC Individual 3	14.6	13.8	97.6	3
15	Low QC Individual 4	15.8	6.1	105	3
15	Low QC Individual 5	14.6	11.6	97.5	3
15	Low QC Individual 6	14.9	4.63	99.4	3
250	Medium QC	247	3.54	99.1	6
4000	High QC	3950	4.59	98.9	6

Solid Phase Extraction: Clobetasol



Solid Phase Extraction: Clobetasol

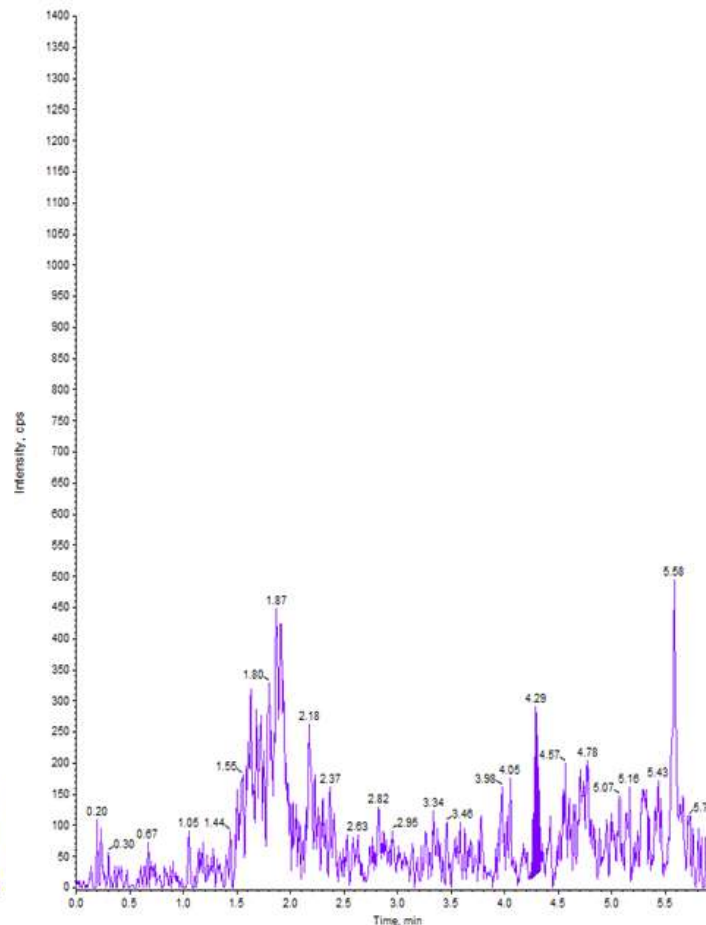
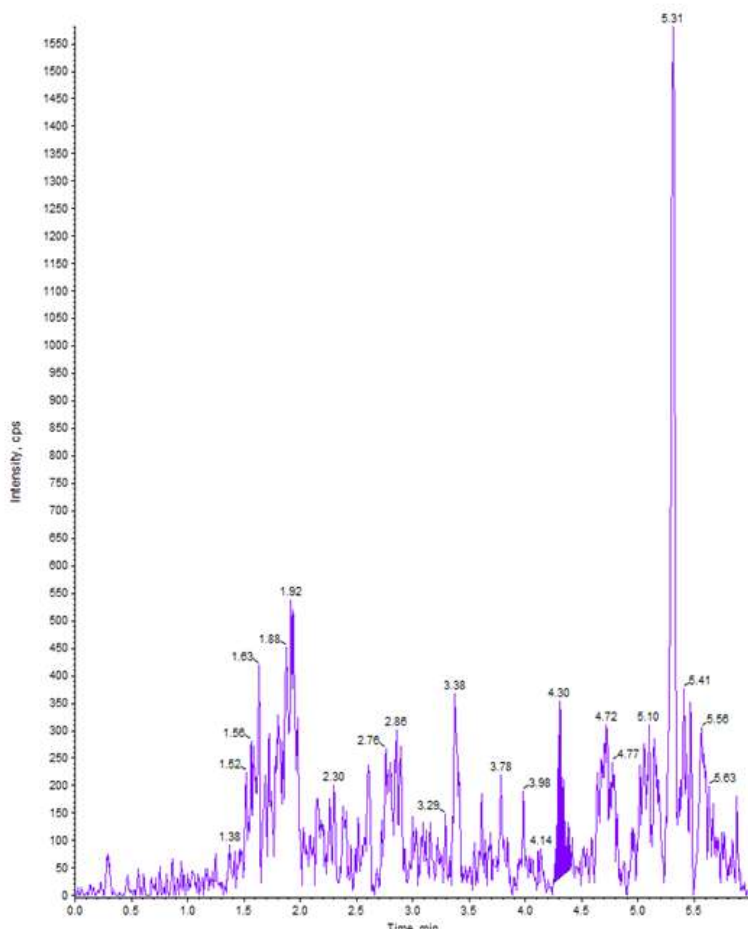
Expected concentration (pg/mL)	Sample name	Mean (pg/mL)	Accuracy %	Number of values
5	Cal 1	4.97	99.3	2
10	Cal 2	9.90	99	2
20	Cal 3	20.8	104	2
50	Cal 4	50.7	101	2
200	Cal 5	198	99	2
800	Cal 6	768	96	2
2200	Cal 7	2180	99.3	2
2500	Cal 8	2540	101.7	2

Passes acceptance criteria

Expected concentration (pg/mL)	Sample name	Mean (pg/mL)	% RSD	Accuracy %	Number of values
5	LLOQ QC	5.22	9.99	104	6
15	Low QC	14.7	7.78	98.2	6
15	Low QC Individual 1	14.4	4.21	95.9	3
15	Low QC Individual 2	14.7	5.44	98.2	3
15	Low QC Individual 3	14.3	4.11	95.4	3
15	Low QC Individual 4	15.7	7.06	105	3
15	Low QC Individual 5	14.3	8.48	95.7	3
15	Low QC Individual 6	13.8	1.63	92.1	3
175	Medium QC	174	3.58	99.4	6
2000	High QC	1970	1.28	98.6	6

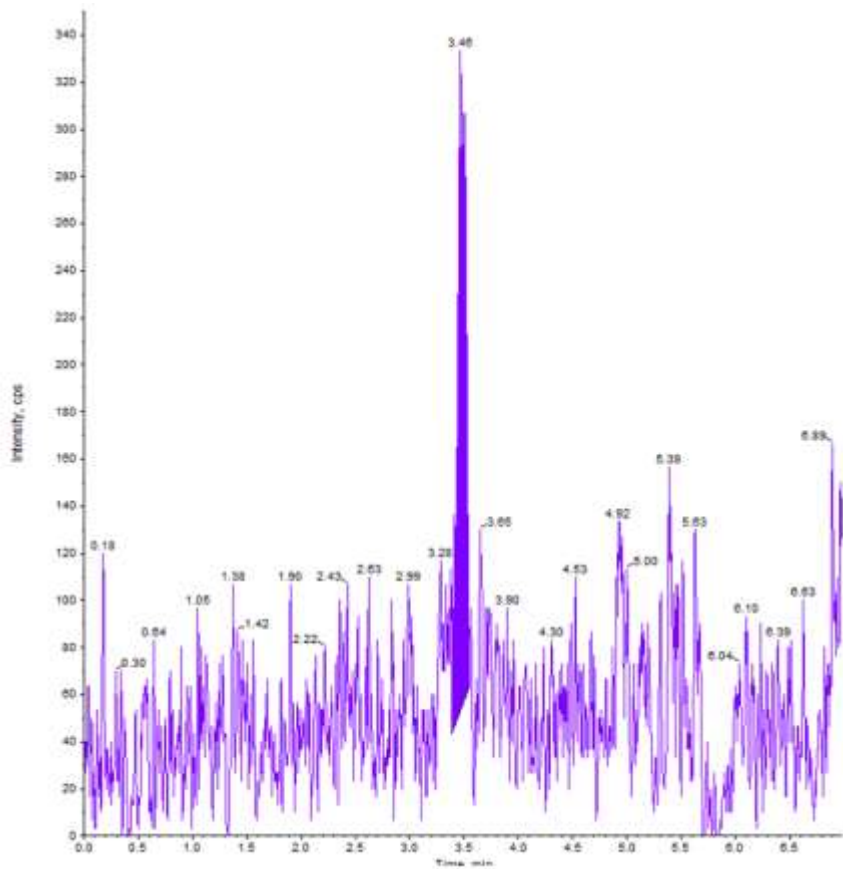
Solid Phase Extraction: Clobetasol-17-Propionate

Interfering peaks causing variability amongst individuals

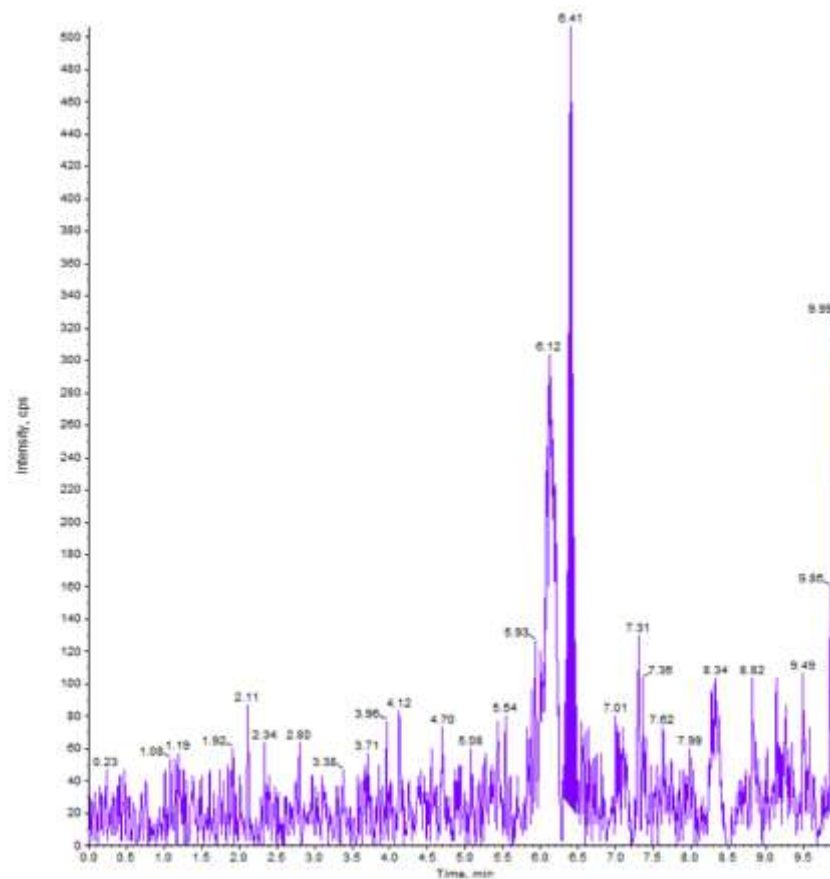


Supported Liquid Extraction Followed by Solid Phase Extraction

Initial chromatography



Optimised chromatography



Conclusion

Two method validated separately:

- ▶ Robust methods
- ▶ Easy to extract
- ▶ Lowest reported limit of quantification
- ▶ Fully validated for clinical study

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