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ANALYTICAL DETERMINATION OF STEROIDAL ADULTERANTS IN HOMEOPATHIC AND HERBAL FORMULATIONS BY LIQUID CHROMATOGRAPHY MASS CHROMATOGRAPHY (LCMS/MS)

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ABSTRACT

A rapid and sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was developed and validated for the determination of steroid residues in homeopathic and herbal drugs. Steroid adulteration in homeopathic and herbal formulations has raised serious safety concerns due to pharmacological effects and regulatory implications. Sample preparation involved solid-phase extraction (SPE) to isolate target analytes, followed by separation using reversed-phase LC with a C18 column. Detection was performed by electrospray ionization in positive ion mode. The method was validated for linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) according to ICH guidelines. The validated method was successfully applied to commercial homeopathic samples. This study demonstrates the applicability of LC-MS/MS for routine quality control of homeopathic and herbal formulations.

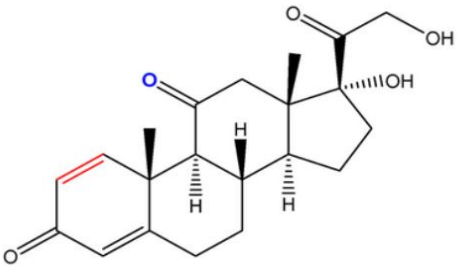
KEYWORDS: Detection was performed by electrospray ionization in positive ion mode.

INTRODUCTION

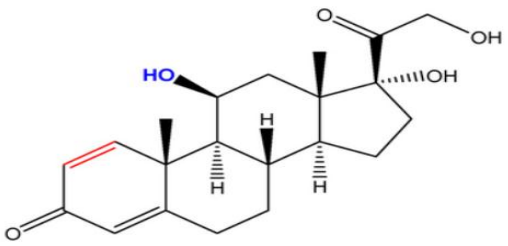
Steroids are pharmacologically active compounds widely used in medicine for their anti-inflammatory and immunosuppressive properties. Recent reports show instances of undeclared steroid presence in homeopathic products, which raises public health concerns and regulatory challenges. Analytical methods capable of separating and quantifying steroid residues at trace levels are essential for ensuring product safety and compliance.

Four steroids Dexamethasone Prednisone and Prednisolone were taken during method analytical method development. Chemical composition and structure of all four steroids is given below. Development.

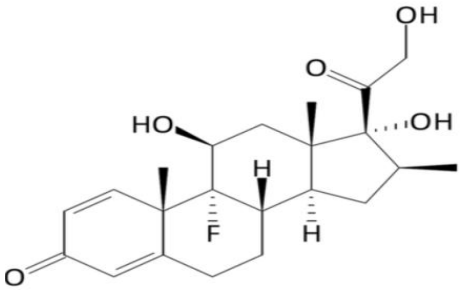
Molecular Structure.



Prednisone



Prednisolone



Dexamethasone

Structural Comparison Summary				
Compound	**C-11**	**C-16**	**C-9**	**Notes**
Dexamethasone	OH	α-CH_3	F	Strong anti-inflammatory
Prednisone	=O	H	H	Prodrug
Prednisolone	OH	H	H	Active metabolite

Molecule formula and molecular weight.

Sr. No.	Compound	Molecular Formula	Molecular Weight (g/mol)
1	Dexamethasone	$C_{22}H_{29}FO_5$	392.46
2	Prednisone	$C_{21}H_{26}O_5$	358.43
4	Prednisolone	$C_{21}H_{28}O_5$	360.44

Homeopathy is a popular alternative medicine system, often marketed as safe and free from synthetic chemicals. Despite this, several studies have reported the presence of undeclared corticosteroids such as prednisolone and dexamethasone in homeopathic preparations. Such adulteration can lead to serious adverse effects, including immunosuppression, metabolic disorders, and dependency.

Instrumentation

Analytical methods are essential to ensure the authenticity and safety of these drugs. LC–MS (Liquid Chromatography–Mass Spectrometry) is particularly suited for this purpose due to its ability to separate complex mixtures and provide accurate mass detection of target analytes. This paper aims to develop and validate an LC–MS method for the determination of steroid content in homeopathic and Herbal formulations.

The C18 (100 × 2.1 mm, 1.7–3 µm) column (Agilent Technologies, Santa Clara, CA, USA) was employed for chromatographic separation. A liquid chromatography (Agilent 1290 infinity) equipped with a triple quadruple mass spectrometer (Agilent Technologies) was used for analysis. Mass spectrometry conditions were Ionization mode (ESI positive), detection parameters (SIM transitions for specific steroids).

Analytical balance, sonicator and vortex also been used during study.

Chemicals

- LCMS-grade methanol
- LCMS grade water
- LCMS grade formic acid.

Standard and sample used

Standards (Steroid)

Prednisone, Dexamethasone and Prednisolone acetate

Sample:

Homeopathic and herbal drug samples sourced from retail pharmacies.

Experiment

Mobile Phase and diluent

A solution of 0.1% (v/v) formic acid prepared in LC–MS grade water served as mobile phase A, whereas 0.1% (v/v) formic acid in LC–MS grade methanol was employed as mobile phase B. Prior to use, both mobile phases were filtered through a 0.22 µm PTFE filter, sonicated to remove dissolved gases, and degassed to ensure chromatographic stability.

LCMS grade Methanol as such used as diluent for standard and test sample.

Standard Solution

Preparation of Standard Stock Solutions

Prednisone Standard Stock Solution (STD STK A)

Accurately weighed and transferred 5 mg of prednisone reference standard was placed into a 10 mL volumetric flask. About 5.0 mL of methanol was added, and the solution was vortexed to achieve complete dissolution. The volume was made up to the mark with diluent and mixed well. This solution contained 500 µg/mL of prednisone and was labelled as **STD STK A**.

Dexamethasone Standard Stock Solution (STD STK B)

Accurately weighed and transferred 10 mg of dexamethasone reference standard was introduced into a 10 mL volumetric flask. Approximately 7 mL of

methanol was added, and the solution was vortexed until complete dissolution. The volume was adjusted to the mark with diluent and mixed thoroughly. The resulting solution contained 1000 µg/mL of dexamethasone and was labelled as **STD STK B**.

Prednisolone Standard Stock Solution (STD STK C)
Accurately weighed and transferred 10 mg of prednisolone reference standard was transferred into a 10 mL volumetric flask. Approximately 7 mL of diluent was added, and the solution was vortexed to ensure complete dissolution. The volume was made up to the mark with diluent and mixed thoroughly. The solution contained 1000 µg/mL of prednisolone and was labelled as **STD STK C**.

Preparation of Mixed Working Standard Solution (STD STK D)
Accurately pipetted 2.0 mL of STD STK A and 1.0 mL each of STD STK B and STD STK C were transferred into a 100 mL volumetric flask. The solution was diluted to volume with diluent and mixed well. The final solution contained 10 µg/mL (10 ppm) each of prednisone, dexamethasone and prednisolone and was labelled as **STD STK D**.

Preparation of Standard Solution for linearity
Standard solutions for linearity were prepared by appropriate dilution of STD STK D with diluent. The resulting solutions contained final concentrations of 0.250, 0.500, 0.750, 1.000, and 1.250 ppm, respectively, for each analyte.

Concentration Level (ppm)	Volume of STD STK D used (mL)	Final Volume with diluent (mL)
0.250	0.25	10
0.500	0.5	10
0.750	0.75	10
1.000	1.0	10
1.250	1.25	10

Sample Preparation: Approximately **1.0 g of the sample** was accurately weighed and transferred into a **10 mL volumetric flask**. The sample was dissolved and diluted to volume with the **diluent**, followed by **thorough mixing** to ensure homogeneity. The resulting solution was then **filtered through a 0.22 µm PVDF membrane filter**.

Chromatographic condition

Column	C18 (150 × 3.0 mm, 1.7–3 µm)
Oven Temperature	40ºC
Injection Volume:	5 µl
Gas Temperature:	300ºC
Flow rate:	0.5 ml/min
Sampler Temperature:	10 0C
Detector:	Mass Detector
Run time:	30 min
Gas Flow	10 L/min
Capillary	3500
Sheath Gas Flow	11 L/Min
Sheath Gas Temperature	280ºC
Nebulizer	45 psi
Ion Source	ESI (+)
Mode	SIM

Compound	Mass	Dwell	Fragmentor	CAV	Polarity
Prednisone	358.28	200	135	5	+
Dexamethasone	392.464	200	135	5	+
Prednisolone acetate	402.487	200	135	5	+

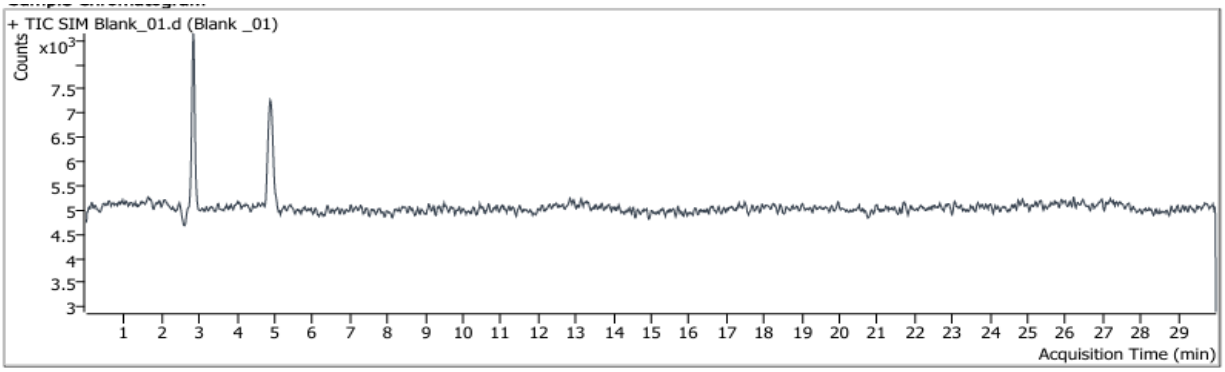
Calculations
The concentration of each steroid in the sample was calculated using the **linearity calibration curve**, which was constructed by plotting **peak area versus concentration (ppm)** of the standard solutions. The **slope and y-intercept** obtained from the linear regression equation were used for quantification.

The concentration of each steroid in the test sample was calculated according to the following formula:

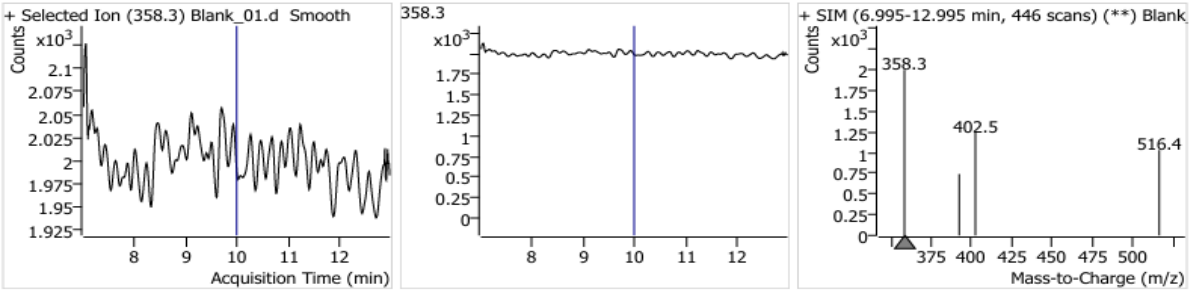
$$\text{Steroid concentration (ppm)} = \frac{(\text{Peak area of steroid in test} - \text{Intercept})}{\text{Slope of linearity curve}} \times \frac{\text{Test dilution}}{\text{Sample weight (g)}}$$

Chromatograms

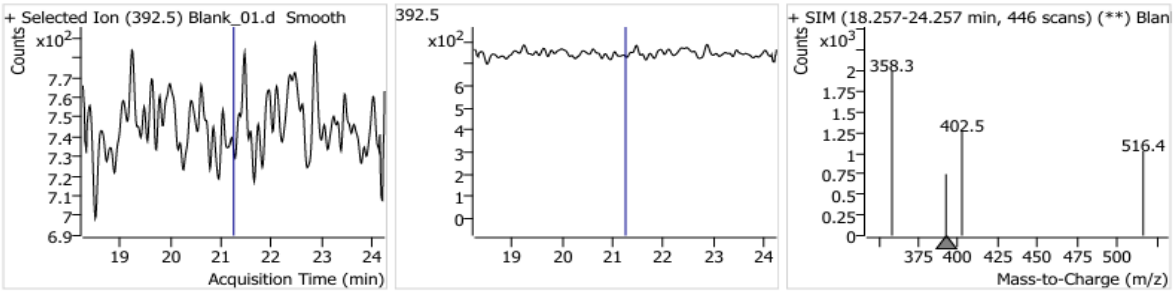
Blank



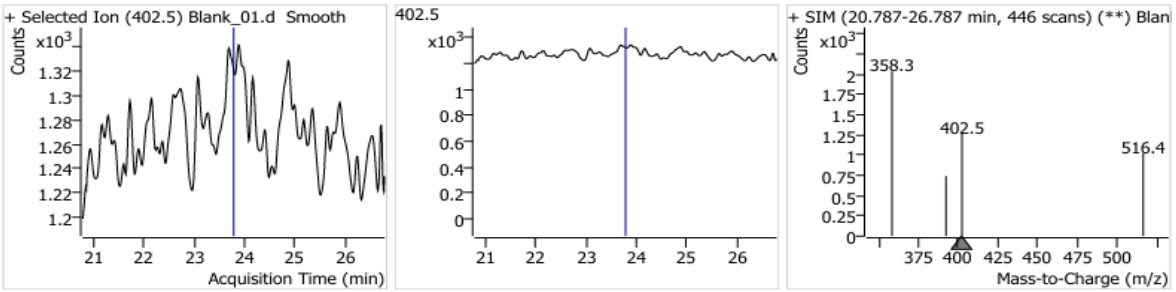
Prednisone



Dexametasone

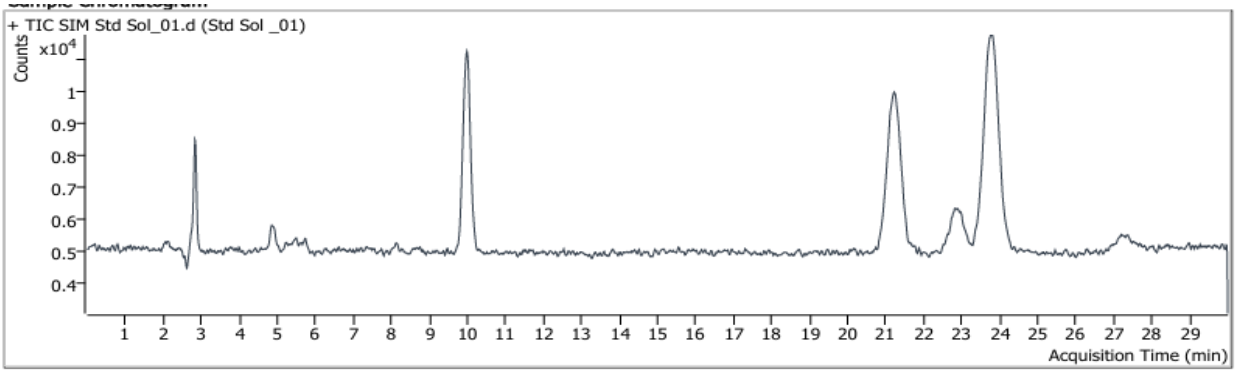


Prednisolone acetate

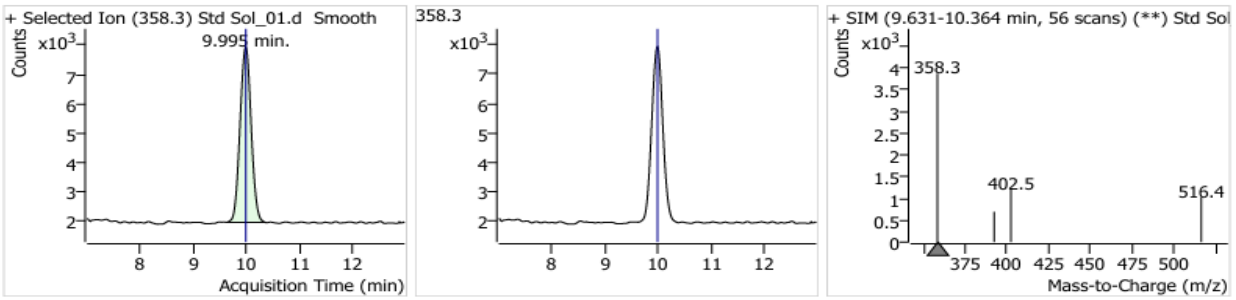


Compound Table				
Compound	**Transition**	**RT**	**Resp.**	**Final Conc.**
Prednisone	358.3			ND
Dexamethasone	392.5			ND
Prednisolone acetate	402.5			ND

Standard



Prednisone



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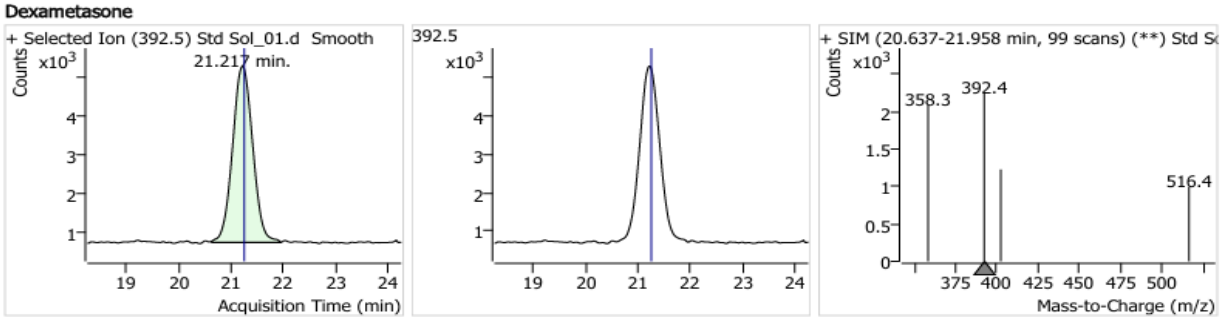
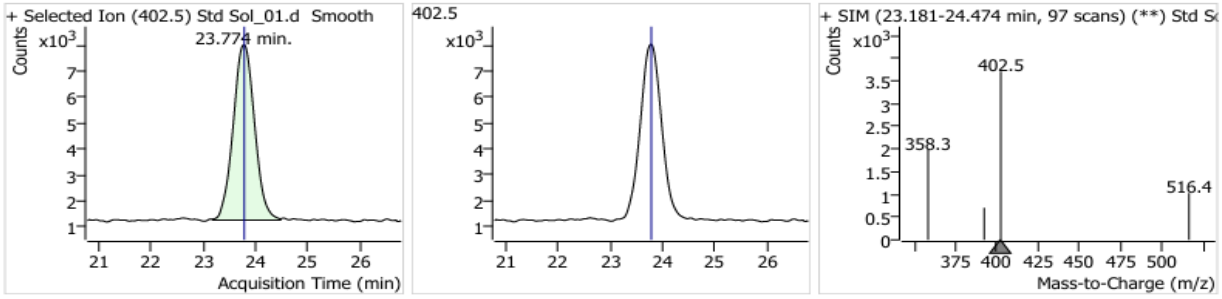
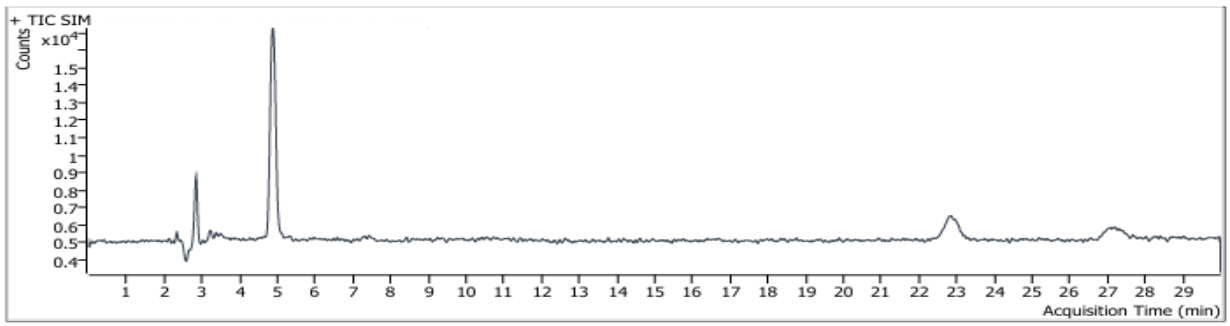


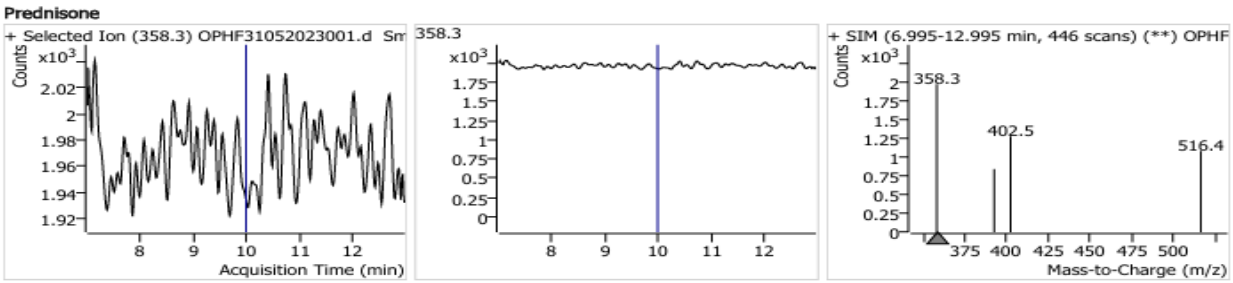
Prednisolone acetate

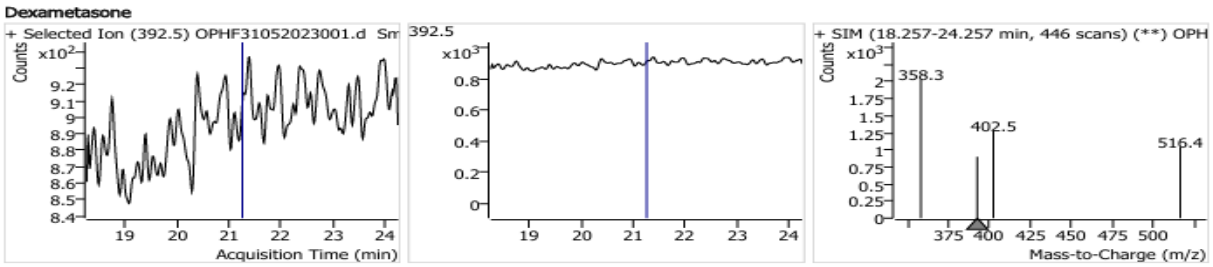


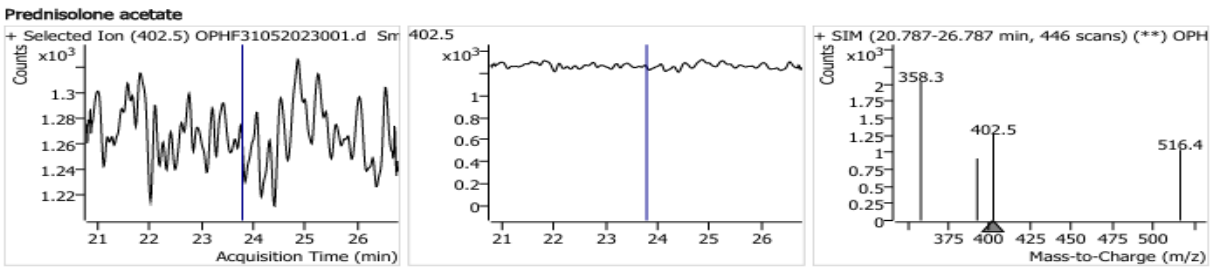
Compound Table				
Compound	**Transition**	**RT**	**Resp.**	**Final Conc.**
Prednisone	358.3	9.995	87343	0.2322
Dexamethasone	392.5	21.217	122845	0.2424
Prednisolone acetate	402.5	23.207	192088	0.2451

Sample







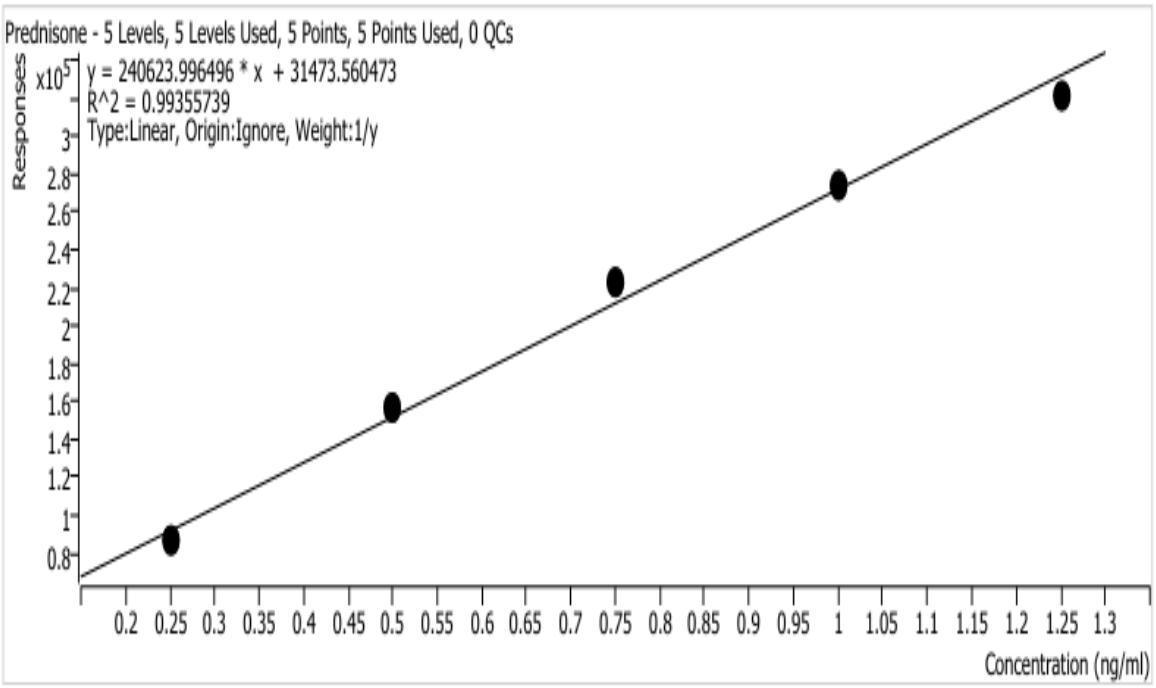


Compound Table					
Compound	**Transition**	**RT**	**Resp.**	**Final Conc.**	**Units**
Prednisone	358.3			ND	ng/ml
Dexamethasone	392.5			ND	ng/ml
Prednisolone acetate	402.5			ND	ng/ml

Linearity
Prednisone

Level	Nominal Concentration	Area Response
1	0.250	87343
2	0.500	156743
3	0.750	222762
4	1.000	273262
5	1.250	320897
Slope		240623.996496
Intercept		31473.560473
Sq. Correlation Coefficient ($R^{2)}$		0.99356

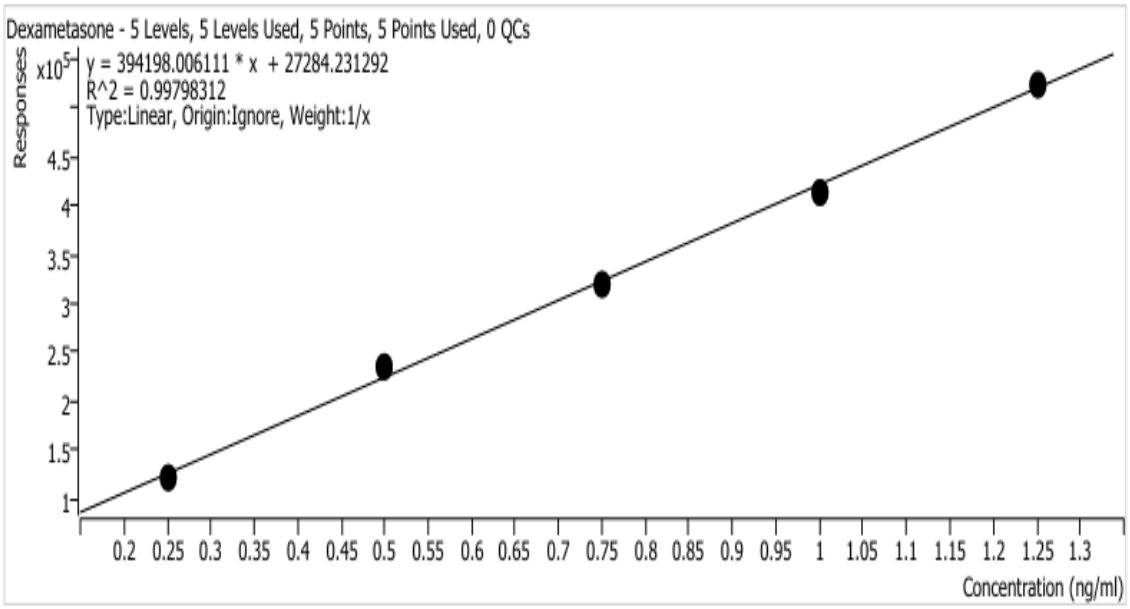
Prednisone



Dexamethasone

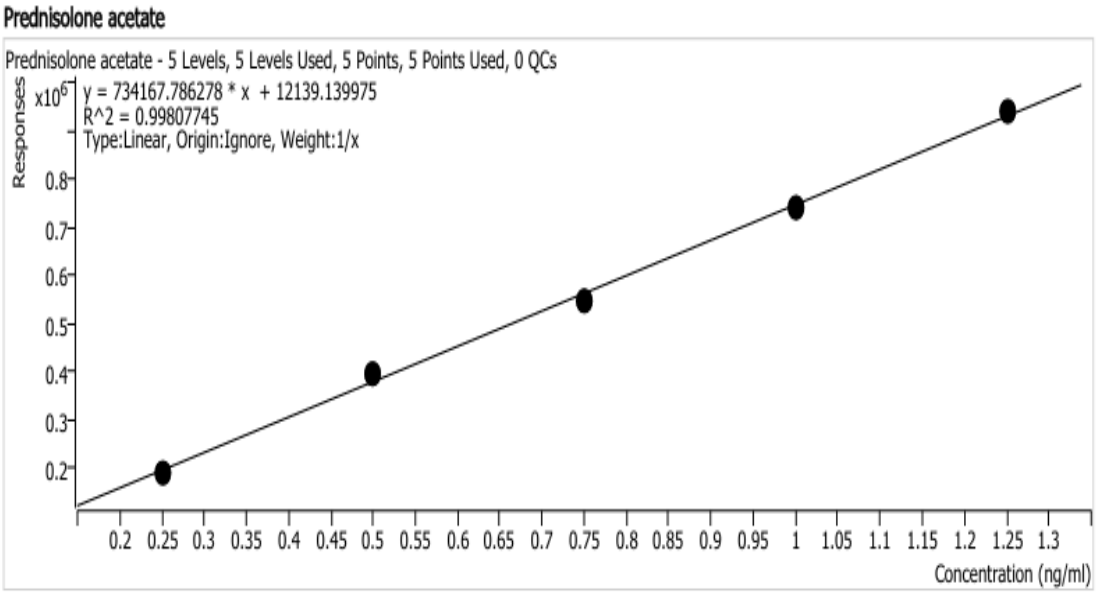
Level	Nominal Concentration	Area Response
1	0.250	122845
2	0.500	234885
3	0.750	319719
4	1.000	414872
5	1.250	522344
Slope		394198.006111
Intercept		27284.231292
Sq. Correlation Coefficient ($R^{2)}$		0.99798

Dexametasone



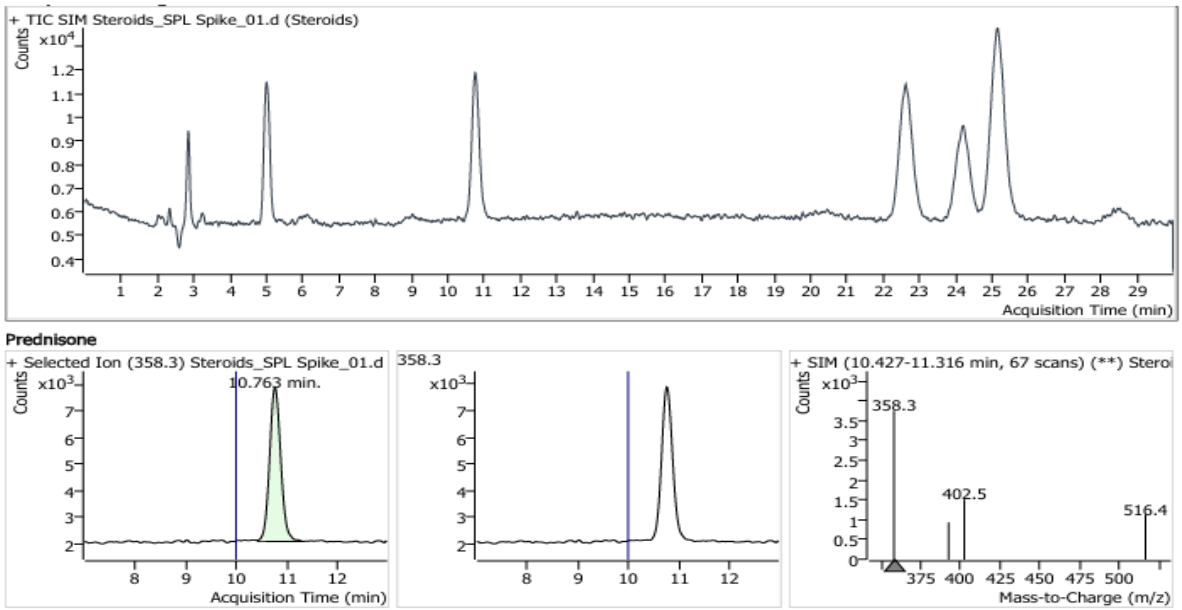
Prednisolone acetate

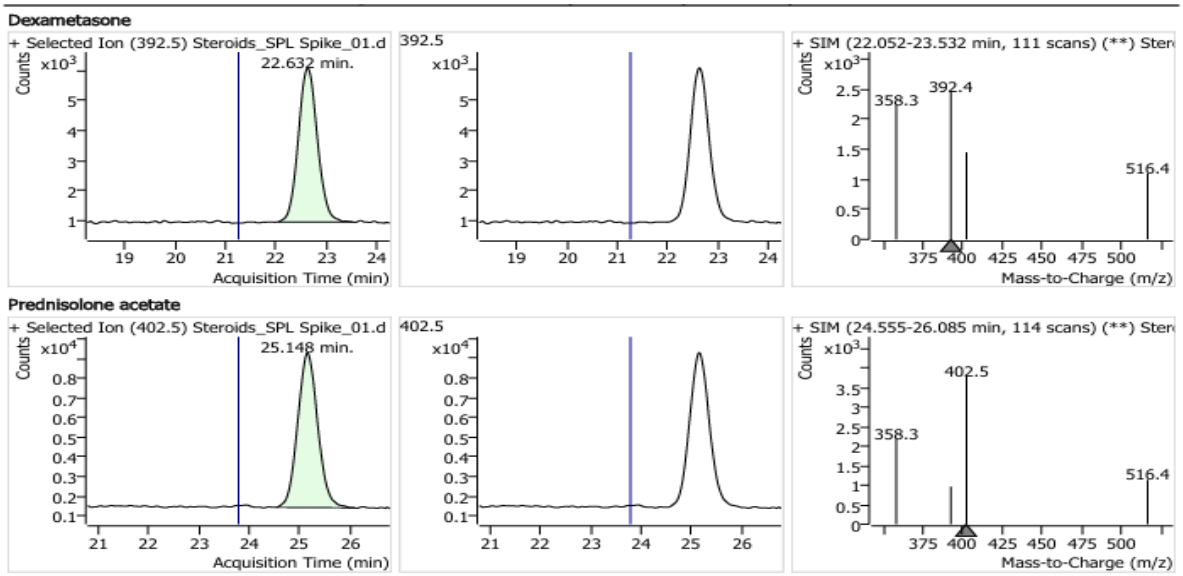
Level	Nominal Concentration	Area Response
1	0.250	192088
2	0.500	396585
3	0.750	547881
4	1.000	739310
5	1.250	937961
Slope		734167.786278
Intercept		12139.139975
Sq. Correlation Coefficient ($R^{2)}$		0.99808



Accuracy (recovery) at LOQ level

Accuracy was performed at LOQ levels.





Compound Table					
Compound	**Transition**	**Amount present in Sample**	**Added Amount (in ppm)**	**Recovered Amount (in ppm)**	**% Recovery**
Prednisone	358.3	ND	0.250	0.2428	97.1
Dexamethasone	392.5	ND	0.250	0.2736	109.4
Prednisolone acetate	402.5	ND	0.250	0.2797	111.9

RESULTS

- Chromatograms showed clear separation of four steroid peaks with retention times between 10–23.5 minutes.
- Clear separation of dexamethasone and betamethasone despite identical m/z.
- Calibration curves exhibited excellent linearity ($R^2 > 0.993$).
- Recovery rates ranged from 80–120%.
- High sensitivity allows detection even in highly diluted homeopathic matrices
- Steroid levels found not detected, indicate **no undeclared adulteration** in the samples have taken for evaluation.
- LC–MS/MS is superior to HPLC-UV for trace analysis

DISCUSSION

The LC–MS method developed here provides a robust and sensitive approach for detecting steroid adulteration in homeopathic drugs. Compared to HPLC–UV, LC–MS offers superior specificity and lower detection limits. The presence of undeclared steroids highlights the need for stricter regulatory oversight and routine screening of alternative medicines.

Challenges include matrix effects from complex herbal formulations, which may require optimized sample preparation. Nevertheless, the method is suitable for routine laboratory application.

CONCLUSION

LC–MS is a powerful analytical tool for detecting steroid adulteration in homeopathic drugs. The validated method ensures high sensitivity, accuracy, and reproducibility, making it ideal for regulatory monitoring and safeguarding public health. Routine application of this technique can help prevent misuse and protect consumers from harmful side effects.

The developed LC–MS/MS method demonstrated excellent sensitivity, selectivity, and reproducibility for the determination of dexamethasone, betamethasone, prednisone, and prednisolone in homeopathic drug formulations and is suitable for routine regulatory surveillance.

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