



## Method Development and Validation of Fludrocortisone Acetate Tablets by Reverse Phase HPLC Method

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### ABSTRACT

The purpose or intent of this current study was to establish a fast and sensitive HPLC technique for the perseverance of Fludrocortisone acetate and utilizing best frequently used HPLC technique. This method had been validated as per the ICH requirements to assure that the method consistently meets the predetermined specifications and quality attributes. Utilizing filtered and degassed pH 3.0 Phosphate buffer and Acetonitrile in the ratio 90:10 as a Mobile phase-A and pH 3.0 Phosphate buffer and Acetonitrile in the ratio 65:35 as a Mobile phase-B the established RP-HPLC technique was done. The separation was achieved by using Waters, X-Bridge Shield RP18, (150 X 4.6-mm), 3.5- $\mu$ m column. Run time and Flow rate was set 45minutes and 1.2mL/min. Injection volume 100 $\mu$ L and wavelength was set 240nm. The correlation coefficient square for fludrocortisones acetate and Fludrocortisone Impurity was found to be 0.9991 and 0.99997. The SD and %RSD for Fludrocortisone Impurity was found to be 0.02 and 1.48 represents method precision. Following validated parameters lies within the limit. Hence, the developed method was precise, simple, fast and accurate.

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### INTRODUCTION

Disorder and then postural hypotension. It is usually consumed along with hydrocortisone in case of adrenal inadequacy and medication is utilized utmost in its acetate form (Elks, 2014; Index Nominum, 2000; Fludrocortisone Acetate, 2016; Day and and, 2010). Fludrocortisone acetate is

an adrenal corticosteroid and has controlled level of glucocorticoid action and has extremely maximum level of mineralocorticoid action. Though it is utilized just for its mineralocorticoid issues. Mineralocorticoid functions in the distal tubules to enhance hydrogen ion evacuation, sodium resorption, to enhance potassium evacuation and later water retention. In further secretory cells cation transfer is equally influenced, evacuation of water by sweat glands and salivary glands is also modified, though to a lower degree. At the cellular levels corticosteroids spread throughout or over cell membranes and intricate with particular or individual cytoplasmic recipient, such complexes afterwards join the cell nucleus attach to DNA and encourage arrangement of mRNA and later protein combination of different enzymes thought to be finally liable for the physiologic issues of such hormones (Lemke and Williams, 2008; Polito *et al.*, 2016). Fludrocortisone is utilized mainly to substitute the lacking hormone aldosterone in diverse forms of adrenal inad-

equacy for instance classic salt squandering form of congenital adrenal hypertrophy and Addison's illness. It has been utilized in the therapy of cerebral salt squandering disorder. Because of its issues on enhancing sodium levels and hence blood volume and then used to heal postural hypotension and to heal low blood pressure (Taplin *et al.*, 2006; Freitas *et al.*, 2000).

### Method Development

Method development is the procedure or method of choosing a precise testing process to establish the constitution of a preparation. Analytical process must be utilized under GLP and GMP conditions and utilizing the customs or properties fixed out in the ICH guidelines the analytical procedure should be established. It is the method of evidence that an analytical procedure is tolerable for employ in laboratory to assess the compactness of consequent specimens. Suitable and quantified or measured instruments, Certified procedure, Change control, Dependable reference standard, Competent analysts, Integrity and Specimen choice (ICH, 2000; European Commission, 2001; Mcdowall, 2005).

## MATERIALS AND METHODS

### Chemicals and reagents

Fludrocortisone Acetate tablets (Equivalent to about 2.5mg) the used pharmaceutical preparation were formulated in-house and used Fludrocortisone Acetate API with a potency 100%. HPLC grade Acetonitrile were purchased from Merck limited and HPLC grade Methanol were purchased from Finar Limited and HPLC grade water. Analytical grade reagents were used.

### Instrumentation

Automated Reverse Phase HPLC equipped with UV-detection chromatographic separation was achieved utilizing Waters, X-Bridge Shield RP18, (150 X 4.6-mm), 3.5- $\mu$ m column. Run time and Flow rate was set 45minutes and 1.2mL/min. Injection volume 100 $\mu$ L and wavelength was set 240nm. For processing data Empower software were used.

The chromatographic Parameters and gradient program are listed in Tables 1 and 2.

From Table 3, developed technique were met the acceptance criteria. Hence the developed method was found to be accurate Tables 4, 5, 6, 7, 8 and 9.

The correlation coefficient square ( $r^2$ ) for Fludrocortisone Acetate and Fludrocortisone Impurity were met the acceptance criteria. The linear regression data shows that the method is linear over the entire concentration range (LOQ (10%)-150%) and

the developed method was found to be precise and accurate Figures 1, 2 and 3.

From Figure 4, developed method were met the acceptance criteria. Hence the developed method was found to be accurate and precise.

Overall average recovery for Fludrocortisone Impurity is between 80.0-120.0%. The %RSD for recovery of triplicate preparations at each level is NMT 10% and hence the method is accurate

No interference was observed from diluents, placebo and all known Impurities at the retention time of Fludrocortisone Acetate peak.

The %difference in area between initial and time points should be NMT 25.0 for Standard. The %difference in %Impurity between initial and time points should be NMT 25.0 for sample.

All results met the acceptance criteria. Based on above results, it is concluded that standard and sample solutions were stable up to 96 hours respectively when stored at Room temperature.

The %difference in Peak area for Impurity between the centrifuged sample and filtered sample should be NMT 25.0.

### Preparation of Buffer

#### Phosphate Buffer pH 3.0

The buffer was prepared by dissolving 2.07 g of sodium di-hydrogen phosphate monohydrate in 1000 mL of water. Mixed well and then the buffer adjusted to pH 3.0 $\pm$ 0.05 with diluted phosphoric acid, then the solution filtered through 0.45  $\mu$ m membrane filter and sonicated the buffer solution to degas.

#### Mobile Phase -A

900mL of pH 3.0 Phosphate buffer and 100mL of Acetonitrile into a suitable container and then sonicated to degas.

#### Mobile Phase-B

650 mL of pH 3.0 Phosphate buffer and 350 mL of Acetonitrile into a suitable container and then sonicated to degas.

### Preparation of Diluent

Used Mobile Phase-B as a diluent.

### Procedure

#### Standard Stock Preparation

50.23mg of Fludrocortisone Acetate RS was weighed and transferred into a 100mL volumetric flask. To that 3/4th volume of diluent was added. Sonicated to dissolve, diluted to volume with diluent and mixed well.

**Table 1: Chromatographic Parameters**

Chromatographic parameters	Conditions/Specifications
Mobile Phase – A	pH 3.0 Buffer: Acetonitrile (90:10,v/v)
Mobile Phase – B	pH 3.0 Buffer: Acetonitrile (65:35,v/v)
Column	Waters, X-Bridge® Shield RP18, (150 X 4.6-mm), 3.5- $\mu$ m
Flow rate	1.2 mL/min
Column Temp.	30°C
Sample Temp.	10 °C
Wavelength	240 nm
Injection Volume	100 $\mu$ L
Run Time	45.0 minutes

**Table 2: Gradient Program**

Time (min)	Mobile Phase-A (%)	Mobile Phase-B (%)
0.00	40.0	60.0
15.00	40.0	60.0
20.00	30.0	70.0
30.00	0.0	100.0
40.00	0.0	100.0
40.10	40.0	60.0
45.00	40.0	60.0

**Table 3: System Precision data**

S. No.	Injection No.	Peak Area for Fludrocortisone Acetate RS	USP Tailing	USP Plate Count
1.	1	151500	1.1	21354
2.	2	154805	1.1	21172
3.	3	152286	1.1	20984
4.	4	152654	1.1	21231
5.	5	153553	1.1	21452
6.	6	154436	1.1	21012
Mean	-	153206	-	-
STDEV	-	1284.65	-	-
%RSD	-	0.8	-	-

**Intermediate Stock Preparation**

Pipetted out 6mL of Fludrocortisone Acetate Standard Stock solution into 100mL volumetric flask. Diluted to volume with diluent and mixed well.

**Standard Preparation**

Pipetted out 2.5mL of Fludrocortisone Acetate Intermediate Stock solution into 50mL volumetric flask. Diluted to volume with diluent and mixed well.

**Preparation of Sample Solution**

Randomly selected the twenty-five (25) tablets and recorded the weight of tablets. Transferred the tablets into a 25mL of volumetric flask. (Equivalent to about 2.5 mg of Fludrocortisone Acetate) added about 15mL of diluent and sonicated the flask until the tablets dispersed. Then spiked the 0.75mL of Fludrocortisone Impurity stock preparation into the same sample solution. Further sonicated to 15 minutes with frequent intermittent shake. After the sonication, diluted to volume with diluent and mixed well. Kept the flask on bench top for about 10 min-

**Table 4: Linearity Data**

Linearity Level (%)	Fludrocortisone Acetate		Fludrocortisone Impurity	
	Concentration ( $\mu\text{g/mL}$ )	Peak Area for Fludrocortisone Acetate	Concentration ( $\mu\text{g/mL}$ )	Peak Area for Fludrocortisone Impurity
LOQ (10%)	0.05	16236	0.150	14978
30	0.151	45752	0.45	45070
50	0.252	77852	0.75	75117
80	0.402	122749	1.2	120188
100	0.503	153206	1.5	151345
120	0.604	185421	1.8	180282
150	0.755	238594	2.25	225353
Correlation Coefficient Square ( $r^2$ )	-	0.9991	-	0.99997

**Table 5: Method Precision Data**

S. No.	Sample Name	Peak Area for Fludrocortisone Impurity	% Impurity
1.	Method Precision-1	150852	1.513
2.	Method Precision-2	151235	1.476
3.	Method Precision-3	150652	1.495
4.	Method Precision-4	151232	1.458
5.	Method Precision-5	152867	1.505
6.	Method Precision-6	153259	1.512
Mean	-	-	1.49
STDEV	-	-	0.02
%RSD	-	-	1.48

**Table 6: Accuracy Data**

Accuracy Levels	Sample #	Peak Area of Fludrocortisone Impurity	Amount Added ( $\mu\text{g/mg}$ )	Amount Recovered ( $\mu\text{g/mg}$ )	% Recovery	Average % Recovery	S.D	%RSD
50%	Sample 1	72382	0.742	0.450	98.9	97.3	1.86	1.9
	Sample 2	70832	0.698	0.732	95.3			
	Sample 3	73135	0.720	0.738	97.6			
100%	Sample 1	150218	1.479	1.506	98.2	98.9	0.97	0.99
	Sample 2	152457	1.501	1.524	98.5			
	Sample 3	153632	1.513	1.512	100.0			
150%	Sample 1	216083	2.128	2.256	94.3	96.5	2.51	2.6
	Sample 2	218753	2.154	2.244	96.0			
	Sample 3	226789	2.233	2.250	99.3			

**Table 7: Solution Stability of Fludrocortisone Acetate at Room Temperature**

Time Interval	Fludrocortisone Acetate Peak Area	% Difference in Area
Initial	105924	N/A
12 Hours	103568	2.22
24 Hours	102559	3.18
48 Hours	101068	4.58
72 Hours	100956	4.69
96 Hours	100582	5.04

**Table 8: Solution Stability of Fludrocortisone Impurity at Room Temperature**

Time Interval	Fludrocortisone Impurity Peak Area	% Difference in Area
Initial	152532	N/A
12 Hours	150980	1.02
24 Hours	150264	1.49
48 Hours	150004	1.66
72 Hours	149925	1.71
96 Hours	149856	1.75

**Table 9: Filter Study Data of Fludrocortisone Impurity**

Sample	Fludrocortisone Impurity Peak Area	% Difference
Centrifuged/Unfiltered	152532	N/A
0.45 $\mu$ m Nylon/2mL discard	153252	0.12
0.45 $\mu$ m Nylon/4mL discard	152117	0.27
0.45 $\mu$ m Nylon/6mL discard	152029	0.33
0.45 $\mu$ m Nylon/8mL discard	151863	0.44

utes and allowed to settle down. Centrifuged the sample for about 5 minutes and collected the supernatant. Filtered the clear aliquot through 0.45- $\mu$ m Nylon syringe filter and collected the filtrate after discarded the first 4mL of filtrate.

#### Preparation of Placebo Solution

Weighed about 2498.12mg of Placebo into 25mL of volumetric flask and added 15mL of diluent and sonicated for 15 minutes with intermittent shaking. Diluted to volume with diluent and mixed well. Centrifuged the sample for about 5 minutes and collected the supernatant. Filtered the clear aliquot through 0.45- $\mu$ m Nylon syringe filter and collected the filtrate after discarded the first 4mL of filtrate.

#### Linearity Procedure

#### Standard Stock Preparation

50.35mg of Fludrocortisone Acetate RS was weighed and transferred into a 100mL volumetric flask. To that 3/4th volume of diluent was added. Soni-

cated to dissolve, diluted to volume with diluent and mixed well.

#### Intermediate Stock Preparation

Pipetted out 5mL of Fludrocortisone Acetate Standard Stock solution into 250mL volumetric flask. Diluted to volume with diluent and mixed well.

#### Standard Preparation

Pipetted out 5mL of Fludrocortisone Acetate Intermediate Stock solution into 100mL volumetric flask. Diluted to volume with diluent and mixed well.

#### Fludrocortisone Impurity Stock Preparation

2.52mg of Fludrocortisone Impurity was weighed and transferred into a 50mL volumetric flask added 35mL of diluent and sonicated to dissolve. After sonication diluted to volume with diluent and mixed well.

#### Fludrocortisone Impurity Preparation

Pipetted out 3mL of Fludrocortisone Impurity stock

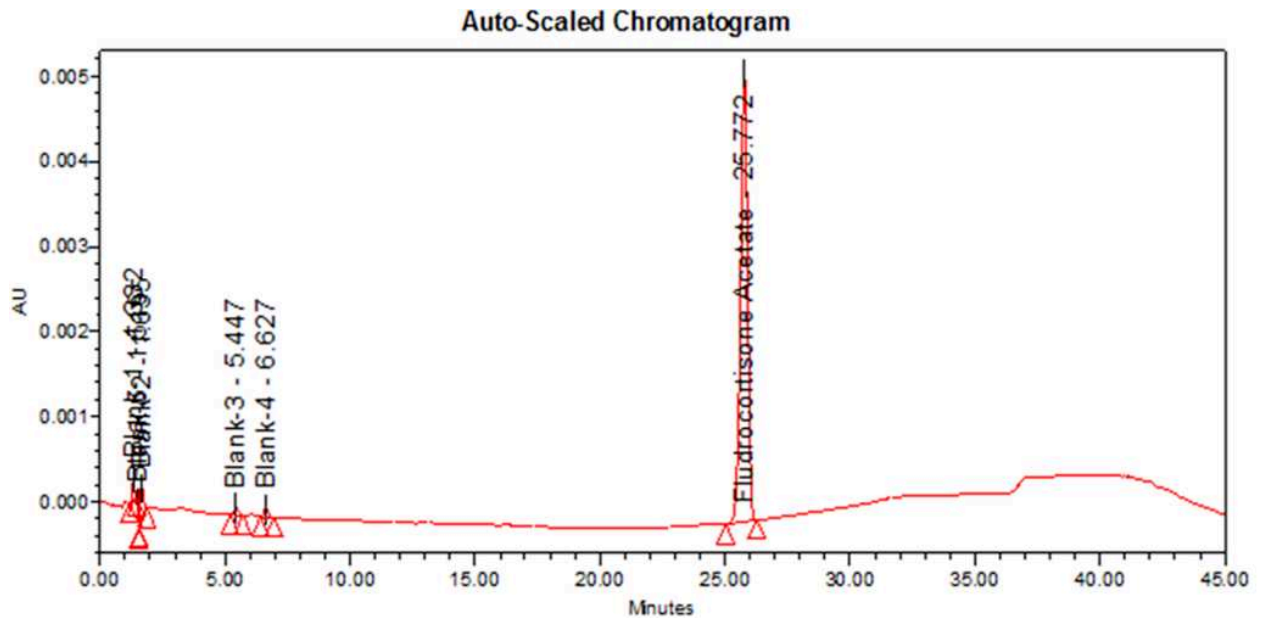


Figure 1: Chromatogram of Fludrocortisone Acetate Standard Injection

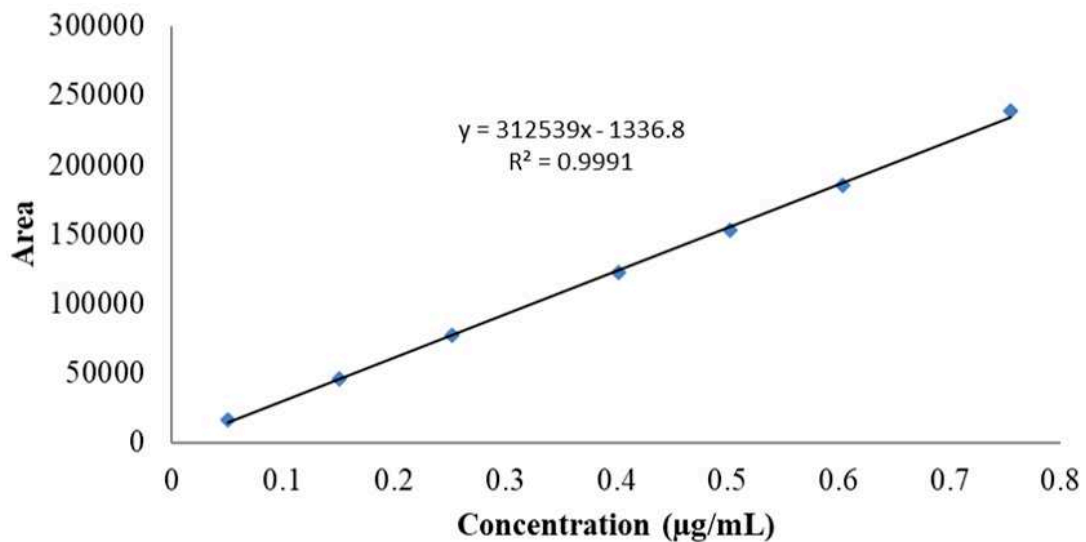


Figure 2: Linearity Graph for Fludrocortisone Acetate

solution into 100mL volumetric flask. Diluted to volume with diluent and mixed well.

#### Initialization of Instrument

By running mobile phase continuously stabilization of chromatographic system and saturation of column is done and baseline graph developed.

#### RESULTS AND DISCUSSION

The %difference in peak area for Impurity between the centrifuged sample and filtered sample was calculated and found less than 25.0. Based on filter study data, it is concluded that samples should be filtered through 0.45µm Nylon filter after discarding first 4mL of filtrate.

The purpose or intent of current study was to establish a fast and sensitive HPLC technique for the perseverance of Fludrocortisone acetate and Fludrocortisone Impurity utilizing best frequently used HPLC technique. The method has been validated as per the guidelines given by ICH requirements to assure that the method consistently meets the predetermined specifications and quality attributes. The system suitability parameter i.e., correlation coefficient square for fludrocortisones acetate and fludrocortisones impurity was found to be 0.9991 and 0.99997. The SD and %RSD for Fludrocortisone Impurity was found to be 0.02 and 1.48 represents method precision. Following validated parameters lies within the limit. Hence the developed method was precise,

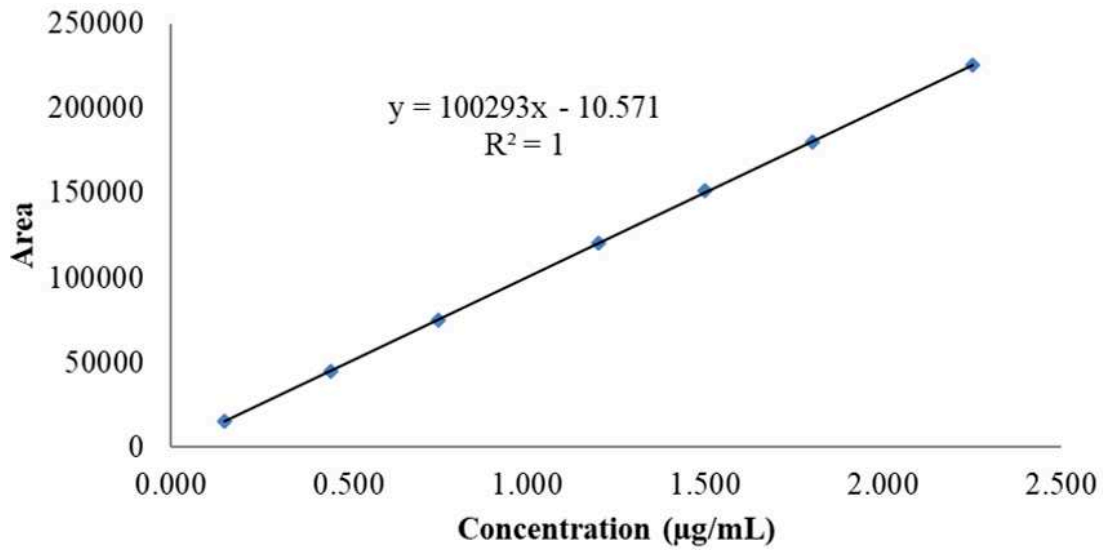


Figure 3: Linearity Graph for Fludrocortisone Impurity

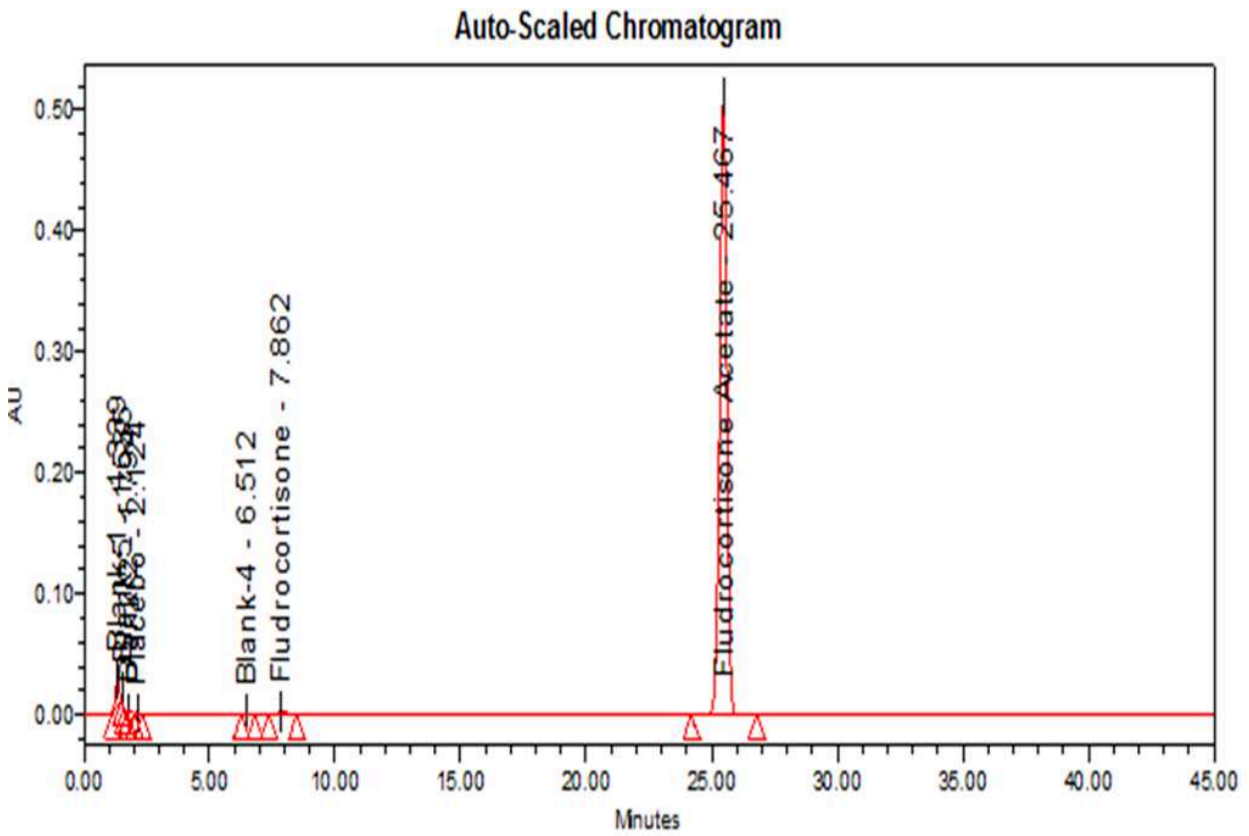


Figure 4: Chromatogram of Fludrocortisone Impurity Sample Injection

simple, fast and accurate.

## CONCLUSIONS

The developed method was validated as per the ICH guidelines instructions. From the determined values it is concluded that the established technique was simple, fast, precise and accurate.

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## Conflict of Interest

The authors declare that they have no conflict of interest for this study.

## REFERENCES

- Day, R. O., and, D. E. F. 2010. Antirheumatic Therapy: Actions and Outcomes. pages 21–21.
- Elks, J. 2014. The Dictionary of Drugs: Chemical Data. *Chemical Data, Structures and Bibliographies*, pages 558–558.
- European Commission 2001. Final Version of Annex 15 to the EU Guide to Good Manufacturing Practice: Qualification and validation. pages 4–5.
- Fludrocortisone Acetate 2016. The American Society of Health-System Pharmacists.
- Freitas, J., Santos, R., Azevedo, E., Costa, O., Carvalho, M., Freitas, A. F. D. 2000. Clinical improvement in patients with bisoprolol and fludrocortisones". *Clinical Autonomic Research*, 10(5):293–299.
- ICH 2000. Technical Requirements for Registration of Pharmaceuticals for Human Use. *Good Manufacturing Practices for Pharmaceutical Ingredients*.
- Index Nominum 2000. International Drug Directory. *Taylor & Francis*, pages 2017–2028.
- Lemke, T. L., Williams, D. A. 2008. Foye's Principles of Medicinal Chemistry. *Lippincott Williams & Wilkins*, pages 2017–2028.
- Mcdowall, R. D. 2005. Effective and Practical risk management options for computerized system validation. *Quality Assurance Journal*, 9(3):196–227.

Polito, A., Hamitouche, N., Ribot, M., Polito, A., Laviolle, B., Bellissant, E., Annane, D., Alvarez, J.-C. 2016. Pharmacokinetics of oral fludrocortisone in septic shock. *British Journal of Clinical Pharmacology*, 82(6):1509–1516.

Taplin, C. E., Cowell, C. T., Silink, M., Ambler, G. R. 2006. Fludrocortisone Therapy in Cerebral Salt Wasting. *Pediatrics*, 118:e1904–e1908.