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RESEARCH ARTICLE

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DETERMINATION OF BECLOMETHASONE DIPROPIONATE, CLOTRIMAZOLE, CHLORAMPHENICOL AND LIDOCAINE IN PHARMACEUTICAL FORMULATIONS USING A NOVEL RP-HPLC METHOD

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ABSTRACT

The aim of this study was to develop a RP-HPLC method for determining the contents of beclomethasone dipropionate, clotrimazole, chloramphenicol and lidocaine in ear drops formulation. Chromatographic separation was achieved on a Octa-decyl silane (C18) column with a simple gradient program, mobile phase is Sol-A: buffer(1.6g of CH_3COONH_2 in to 1000mL of HPLC grade water, add 10mL of TEA and adjust the pH to 6.4± 0.1 with diluted acetic acid) and Sol-B: Acetonitrile. 1.0ml per min flow rate and detection was at 254 nm. Chloramphenicol, lidocaine, belclomethasone dipropionate and clotrimazole were eluted at 5.0min, 10.8min, 16.2min and 19.1min, respectively. The absorbance of four active peaks was a linear function of concentration in the range 1.0ppm to 6.0ppm for Beclomethasone Dipropionate, 187.5ppm to 1125.0ppm for Chloramphenicol, 37.5ppm to 225.0ppm for Clotrimazole and 65ppm to 390ppm for Lidocaine, correlation coefficients of Beclomethasone Dipropionate is 0.9997, Chloramphenicol is 0.9999, Clotrimazole is 0.9998 and Lidocaine is 0.9999 respectively. The reverse-phase HPLC assay method was developed and validated. The method has wide applicability for these four active ingredients. The method has reproducibility, accuracy and robust.

KEYWORDS

Beclomethasone dipropionate, chloramphenicol, clotrimazole, lidocaine, ear drops and RP-HPLC method.

INTRODUCTION

Beclomethasone [1-4] is a glucocorticoid steroid. It is used as an inhaler; the medication goes directly into the lungs, and very little amount finds its way into the rest of the body. Higher doses of inhaled beclomethasone (more than 1000 mcg/day) may result in more absorption into the body. This may decrease bone formation and increase bone breakdown (resorption), resulting in weak bones and a risk of fractures. Even higher doses (more than 1500 mcg/day in adults and 400mcg/day in children) may suppress the adrenal glands and impair their ability to make natural glucocorticoid. People with such suppression (which can be identified by testing) need increased amounts of glucocorticoid orally or by the intravenous route during periods of high physical stress; since higher amounts of glucocorticoids are needed by the body to fight physical stress.

Chloramphenicol [5-11] is an antibiotic drug, used to treat a variety of bacterial infections. It works by killing bacteria or preventing their growth. Chloramphenicol should not be used for colds, flu, other virus infections, sore throats or other minor infections, or to prevent infections. It may cause some serious side effects, including blood problems and eye problems. Symptoms of the blood problems include pale skin, sore throat and fever, unusual bleeding or bruising and unusual tiredness or weakness. Clotrimazole [12-14] is an antifungal drug and used in the treatment of fungal infections of both humans and other animals such as vaginal yeast infections, oral thrush and ringworm, athlete's foot and jock itch. It can also be used to prevent oral thrush. The most commonly noted side effects are redness, stinging, blistering, peeling, swelling, itching, hives, or burning at the area of application. Lidocaine [15-19] is a common local anesthetic and antiarrhythmic drug and used to treat itching, burning and pain from skin inflammations. Lidocaine patches are used to relieve the pain of post-herpetic neuralgia (the burning, stabbing pains or aches that may last for months or years after a shingles infection). Chemical structures of all active ingredients were represented in figure-1.

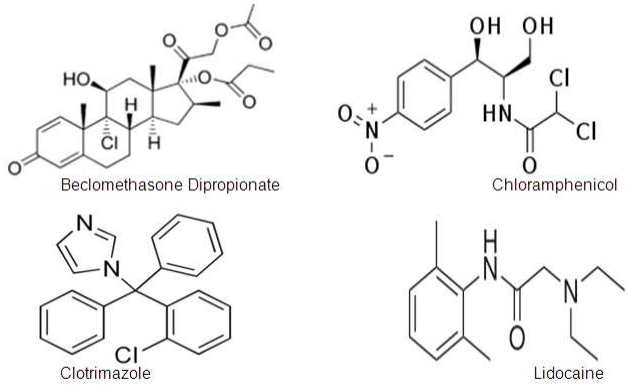


Figure-1
Chemical structure of all active materials.

All four active ingredients have individual and combination dosage forms and have methods [20-29]. The main objective of this research work is to develop a single RP-HPLC method the simultaneous determination of all four active ingredients.

MATERIALS AND METHODS

Reagents and materials

All purified standard materials were used for this study. Acetonitrile, acetic acid, tri-ethyl amine were obtained from Fisher Scientific. HPLC grade water used for analysis (Merck India Limited). All market samples were purchased and analyzed with the developed method.

Instrumental and analytical conditions

The HPLC system consisted of Waters alliance 2695 auto sampler and Waters 2487 dual λ absorbance detector controlled by Waters Empower-2 software. Separation was achieved with acetate buffer (1.6gm of ammonium acetate in to 1000 mL of HPLC water, add 10mL of Trimethylamine, adjust the pH to 6.4 with diluted acetic acid and degas with 0.45µ filter) and acetonitrile with simple gradient program (0-4 min 35% CH_3CN; 4-8 min linear from 35% CH_3CN to 65% CH_3CN; 8-15min 65% CH_3CN; 15-20min linear from 65% CH_3CN to 45% CH_3CN; 20-22min linear from 45% CH_3CN to 35% CH_3CN; 22-25min linear from 35% CH_3CN) with a reversed phase C18 column (250 mm x 4.6mm, 5.0 µ). 1.0ml per min flow rate and 20 µL Injection volume was selected. Response was a linear function of concentration in the range 168ppm to 504ppm for Beclomethasone Dipropionate and 11ppm to 33ppm for Chloramphenicol, 11ppm to 110ppm Clotrimazole and 22ppm to 23ppm Lidocaine;

Diluent:

Mixed the buffer and Acetonitrile in the ratio of 1:1 and degassed.

Preparation of Standards:

A. Standard stock solution:

Weigh accurately 40mg of Beclomethasone dipropionate working standard in to a 100ml volumetric flask, add 75mL of diluent, and sonicate to dissolve then dilute to volume with diluent.

B. Standard solution:

Weigh accurately 75mg of Chloramphenicol working standard, 26mg of Lidocaine working standard and 15mg of Clotrimazole working standard in to a 100ml volumetric flask, add 75mL of diluent and add 1.0ml of Beclomethasone standard stock solution and sonicate to dissolve, dilute to volume with diluent.

Test preparation:

Prepare the test solution equivalent to beclomethasone dipropionate-1.0ppm, chloramphenicol-750ppm, clotrimazole-150ppm and lidocaine-260ppm with diluent.

System Suitability:

The %RSD of five replicate standard injections is not more than 2.0% and tailing factor of standard peaks is not more than 2.0.

Percentage (%) of active content

$$= \frac{At \times CS \times P}{As \times CT}$$

Where in As is the area of standard solution, At is the area of test sample solution, CS is the concentration for standard preparation, CT is the concentration of active ingredient in test solution and P is the Potency of standard.

RESULTS AND DISCUSSIONS

Method development:

Method development trials were performed with phosphate and perchlorate buffer salts by using C18, 250mm column but the separation was not achieved and finally method has been optimized. The retention time of Chloramphenicol is 5.9min, Lidocaine is 10.8min, Beclomethasone dipropionate is 16.2min and Clotrimazole is 19.1min, respectively. Figure-2 represents the standard chromatogram. Perfect base line separation was achieved with acetate buffer and acetonitrile with a simple gradient

program. Five replicated injections of the sample shows that the area of Beclomethasone, Chloramphenicol, Clotrimazole and Lidocaine are consistent in all the injections and passes the system suitability (%RSD) of not more than 2%. Table-1, 2 and 3 represents the system suitability results.

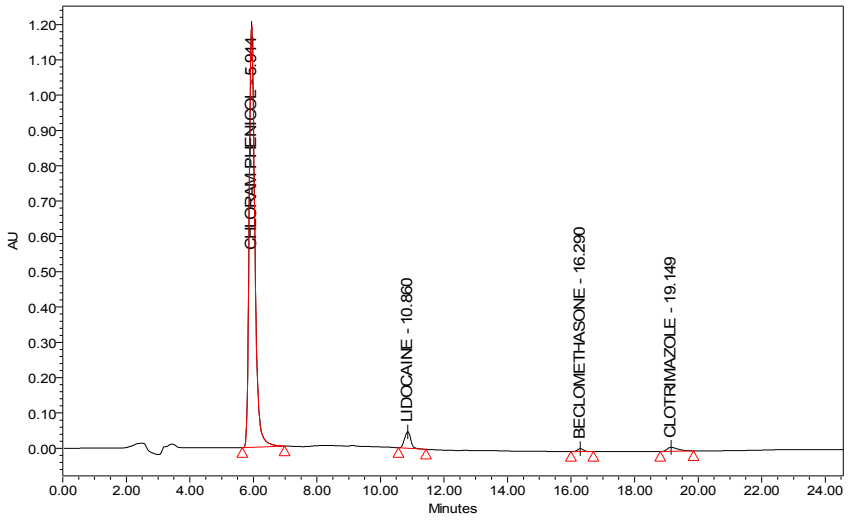


Figure-2

Standard solution of Beclomethasone, Chloramphenicol, Clotrimazole and Lidocaine.

Table-1

%RSD for retention time of all active ingredients

Standard solution	Retention time (min)			
	Chloramphenicol	Lidocaine	Beclomethasone	Clotrimazole
Standard-1	5.94	10.86	16.29	19.14
Standard-2	5.91	10.86	16.25	19.07
Standard-3	5.92	10.88	16.23	19.01
Standard-4	5.90	10.91	16.25	19.05
Standard-5	5.90	10.90	16.20	18.98
Average	5.91	10.88	16.24	19.05
%RSD	0.28	0.20	0.20	0.32

Table-2

%RSD for standard solution area of all active ingredients

Standard solution	Peak area			
	Chloramphenicol	Lidocaine	Beclomethasone	Clotrimazole
Standard-1	14893246	629570	1014105	278518
Standard-2	14517517	616799	1002914	275691
Standard-3	14895344	617533	996936	271971
Standard-4	14861819	616387	1002291	278570
Standard-5	14897540	613932	1021217	278976
Average	14813093	618844	1007493	276745
%RSD	1.12	0.99	0.98	1.07

Table-3
Tailing factor of all active ingredients

Standard solution	Peak area			
	Chloramphenicol	Lidocaine	Beclomethasone	Clotrimazole
Standard-1	1.34	1.22	1.19	1.26
Standard-2	1.35	1.38	1.17	1.22
Standard-3	1.36	1.28	1.19	1.24
Standard-4	1.38	1.28	1.28	1.29
Standard-5	1.39	1.30	1.31	1.29
Average	1.36	1.29	1.22	1.26

Method validation:

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by performing six preparations, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on three different days (inter-day) and also by another analyst performing the analysis in the same laboratory (between-analysts).

Linearity and range

The range of linearity was determined by constructing calibration curves for four active ingredients. Before injection of linearity solutions, the system was equilibrated for 30 min with mobile phase. Concentration range for Beclomethasone dipropionate is 1.0ppm to 6.0ppm, for Chloramphenicol is 187.5 to 1125.0ppm, for Clotrimazole is 37.5ppm to 225.0ppm and for Lidocaine it is 65.0ppm to 390.0ppm, respectively. The correlation coefficient values found to be within the limit. Figure-3A and 3B represents the linearity chromatograms. Linearity results and graphs were tabulated in table-4, 5, 6 and 7.

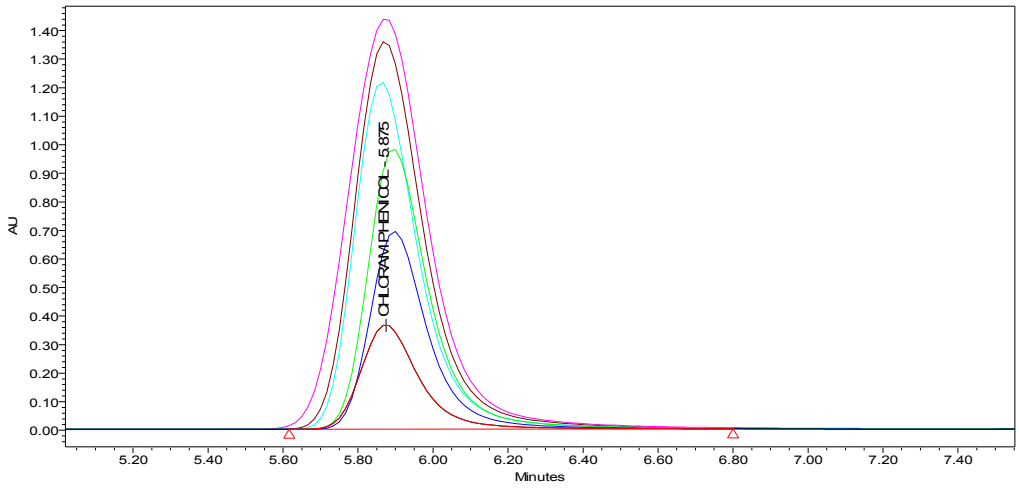


Figure - 3A
Linearity of Chloramphenicol at retention time of 5.87min

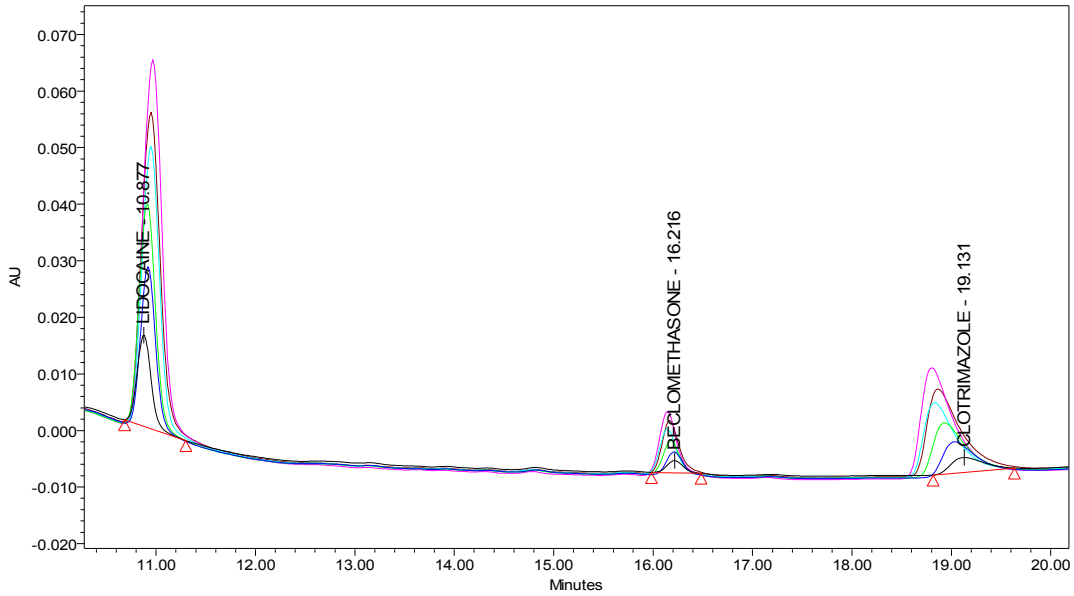


Figure-3B
Linearity of Beclomethasone, Clotrimazole and Lidocaine.

Table-4
Linearity graph of Beclomethasone

Beclomethasone Dipropionate Linearity		
Linearity Level	**Concentration (ppm)**	**Area**
1	1	25121
2	2	50210
3	3	74315
4	4	96086
5	5	121802
6	6	142902
Correlation co-efficient		0.9997

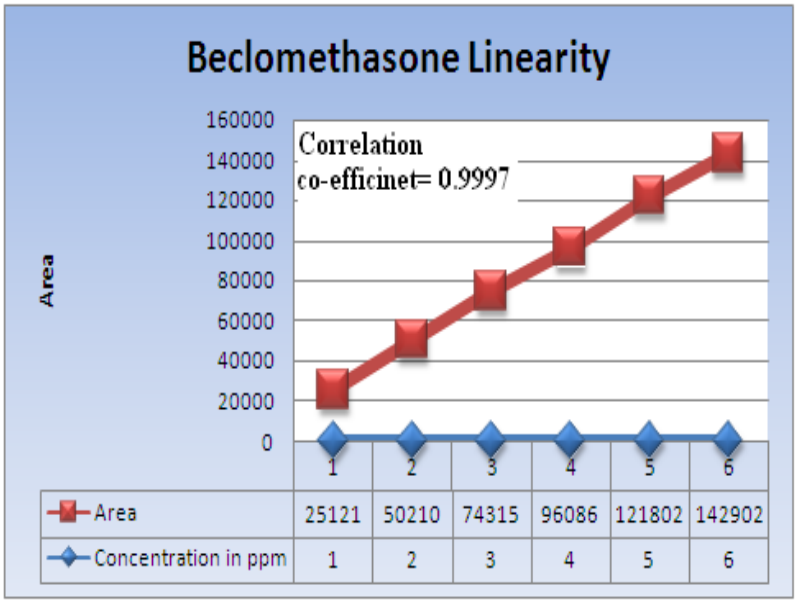


Table-5
Linearity graph of Chloramphenicol

Chloramphenicol Linearity		
Linearity Level	**Concentration (ppm)**	**Area**
1	187.5	3657185
2	375	7314361
3	562	10861801
4	750	14528738
5	937.5	18485923
6	1125	21843107
Correlation co-efficient		0.9999

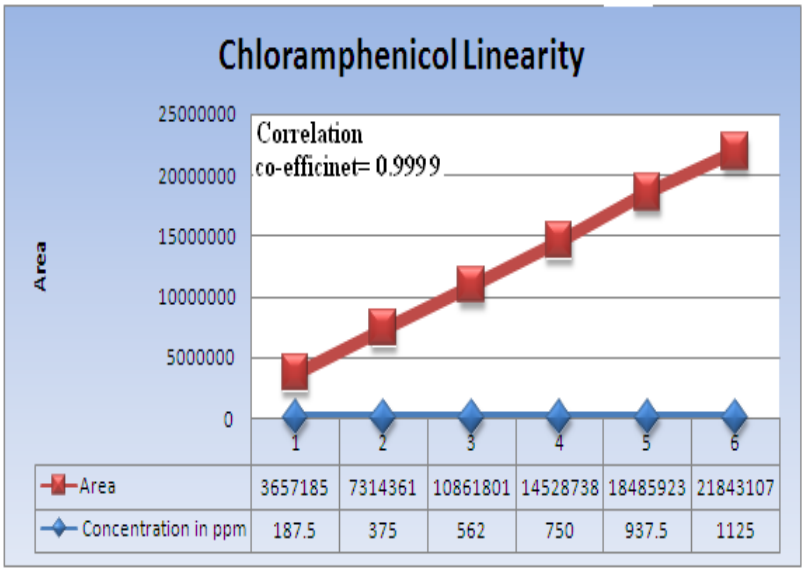


Table-6
Linearity graph of Clotrimazole

Clotrimazole Linearity		
Linearity Level	**Concentration (ppm)**	**Area**
1	37.5	69615
2	75	140207
3	112.5	211792
4	150	281413
5	187.5	355016
6	225	418620
Correlation co-efficient		0.9998

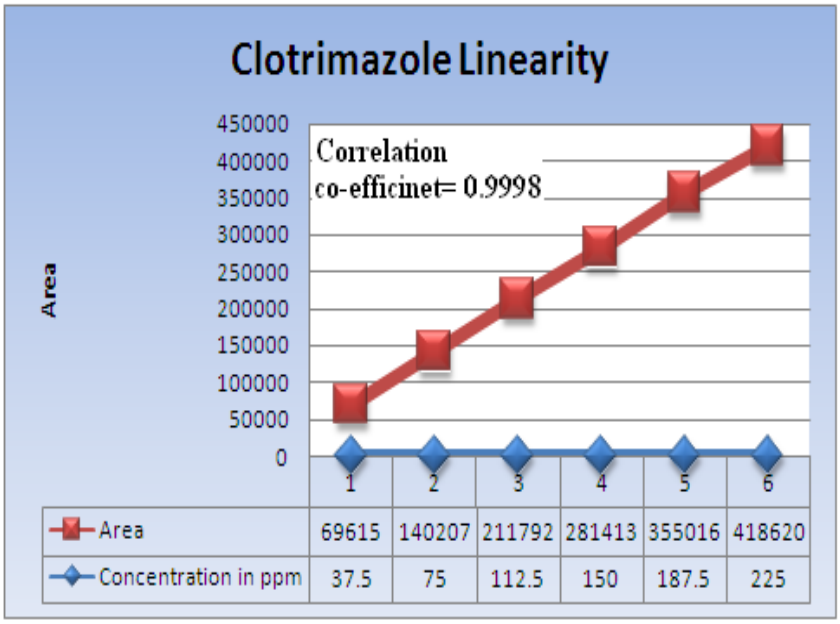
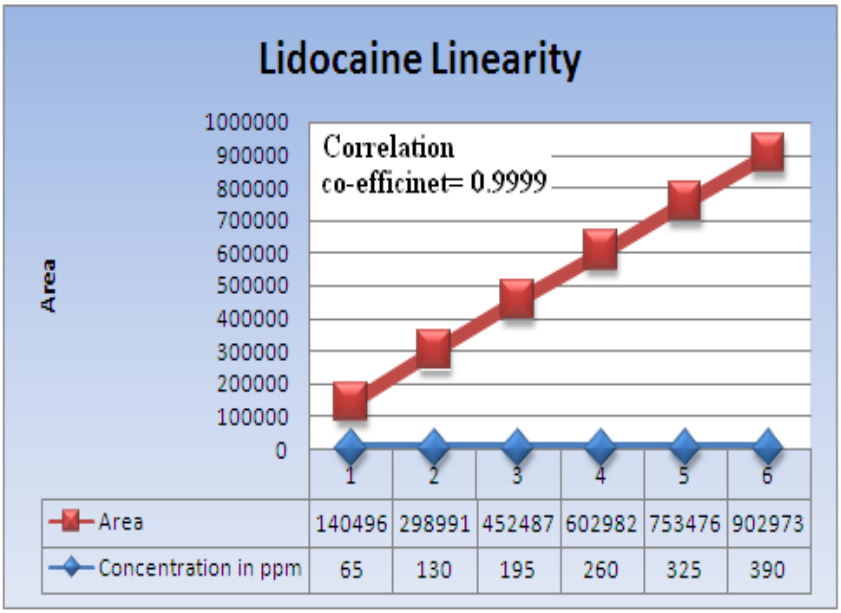


Table-7
Linearity graph of Lidocaine

Lidocaine Linearity		
Linearity Level	**Concentration (ppm)**	**Area**
1	65	140496
2	130	298991
3	195	452487
4	260	602982
5	325	753476
6	390	902973
Correlation co-efficient		0.9999



Accuracy

To confirm the accuracy of the proposed method, a total of 30 determinations were performed using six concentration levels covering the specified range. Accuracy results found to be within the limit (between 98% to 102% recovery).

Robustness and Ruggedness:

The ruggedness of the method was determined by carrying out the experiment on different instruments like waters make HPLC and shimadzu makes HPLC by different analysts using different brand new columns of similar types. The percent RSD of six different preparations assay values with two different instruments, analysts and columns were 1.5- 1.2, 1.6- 1.1 and 0.4- 1.3% respectively.

The robustness was assessed by altering the chromatographic conditions such as, by changing the flow rate, column temperature and the results found to be good. Robustness results were tabulated in table-8.

Table-8
Robustness Results.

Parameter	System suitability		
	Variation	**Tailing factor**	**Percent (%) RSD**
Standard solution	-----	1.1-1.3	0.9-1.1
Flow Rate	+0.1mL per min	0.9-1.2	0.8-1.2
	-0.1mL per min	1.2-1.5	0.8-1.0
Column Oven Temperature	+5°C	1.2-1.4	1.0-1.2
	-5°C	1.1-1.5	1.1-1.3

CONCLUSION

An HPLC-UV method for determination of Beclomethasone dipropionate, Chloramphenicol, Clotrimazole and Lidocaine in ear drops was developed and validated as per ICH and FDA guidelines. Linearity, accuracy, ruggedness and robustness results reveal that the proposed method is accurate, precise and robust. This method was applicable for regular quality control analysis.

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