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Spectroscopic and Chromatographic Method Development and Validation of Sodium Cromoglycate in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The simple, accurate & precise UV-Visible spectroscopic method has been developed and validated for quantitative determination of Sodium Cromoglycate. The present paper reported a highly selective & robust RP-HPLC method, validated for determination of Sodium Cromoglycate dosage form. The experimental design describes the key of HPLC method components including Mobile phase. Sodium cromoglycate is chemically (Disodium5-(3-(2-carboxylato-4-oxocromen-5-yl) -oxy-2-hydroxypropoxy-) -4-oxocromen-2-carboxylate). A rapid, specific & economic UV-Visible spectroscopic method has been developed using a solvent Acetonitrile to determine sodium cromoglycate in bulk & pharmaceutical dosage formulations. The quantitative determination of the drug has been carried out at a predetermined λ Max 272 nm, it was provided linearity in a range 2 -10µg/ml & exhibited good correlation coefficient (R^2 = 0.9899) & excellent recovery (92.83 – 102.16%) and simple & accurate reverse phase liquid chromatography (RP-HPLC) method was developed for the quantitative estimation of sodium cromoglycate. The drug was on a reverse phase C-18 column. Elements were monitored at wavelength of 272 nm using a mixture of 0.1 % perchloric acid: acetonitrile (80:20v/v). The retention time of sodium cromoglycate was found to be 4.59 minutes. The flow rate of mobile phase was 1ml /min at R.T. The percentage recoveries lies in the range of 99.75%.

***Keywords**: Sodium Cromoglycate (SCG), RP-HPLC, FT-IR, R^2.*

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INTRODUCTION

High-performance liquid chromatography (HPLC) is an advanced form of liquid chromatography used in separating complex mixture of molecules involved in chemical and biological systems. It has become the principal method in USP (United States Pharmacopoeia) and to a lesser extent of the most widely used methods also in The European Pharmacopoeia.(1)

Sodium cromoglycate (NaCr), *di-sodium 4,4'-dioxo-5,5'-(2-hydroxytrimethylenedioxy) di(4Hchromene-2-carboxylate)*, is believed to act primarily by preventing release of mediators of inflammation from sensitized mast cells through stabilization of mast cell membranes. It is used in the prophylaxis of many types of asthma i.e. chronic asthma or acute asthma in children as well as adults also. The official methods for the determination of NaCr are non aqueous titration using standard perchloric acid as titrant. (2)

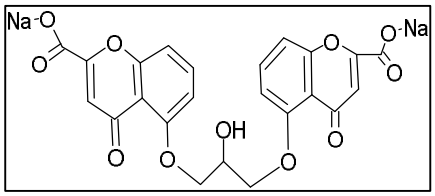

Fig. 1 : Structure of Sodium Cromoglycate

The drug is available for use in dry powder inhalers (DPI), metered dose inhalers (MDI) nebulizers and ophthalmic preparations. (3) The official methods include non aqueous titrations and Spectrophotometry

in USP. The drug has been also determined by TLC densitometric methods and also; electrochemical methods. (4) Several HPLC methods were described for the determination of SCG using UV detection, fluorescence detection or tandem mass spectrometric detection and by simple spectrophotometric methods.(5)

MATERIALS AND METHODS

✓ Chemicals and Materials

Sodium cromoglycate API (SCG)	Rakshit Pharmaceutical Ltd. Mumbai.
Water	Spectroscopic and Chromatographic grade
Perchloric acid 0.1%	Laboratory Grade
Acetonitrile	HPLC grade

✓ Equipment's

Spectroscopic work was performed by using Shimadzu 1800. Chromatographic separation was carried out using a Agilent 1260-infinity -2 equipped with a Rheodyne injector valve with a 20 µL loop and an DAD detector having Column Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ). Mobile phases were filtered using 0.45 µm membrane filters (Millipore, Cork, Ireland) and degassed using a prominence degasser.

✓ Preparation of solutions

1. For UV-Spectroscopy

Standard Stock Solution preparation (SSS-1) [6]

Initially prepare a Standard Stock Solution (SSS-1) of Sodium Cromoglycate by adding 5mg in 10 ml volumetric flask & add 5 ml diluent (Acetonitrile:Water = 80 :20) and mix, sonicate for 5 minutes. Make up the volume upto 10 ml with diluent. (Conc. = 500 µg/ml)

Pipette out 1.0 ml of SSS-I in 10 ml volumetric flask. Add 5 ml diluent (Acetonitrile :Water = 80 : 20) and vortex; make up the volume with diluent, Conc. = 50 µg/ml is the final Stock Solution.

Standard Test Solution Preparation

From the above stock solution pipette out 0.4ml, 0.8ml, 1.2ml, 1.6ml, 2.0ml and make up the volume up to 10 ml for each sample with diluents (Acetonitrile : Water = 80 : 20) to form final solution, having Conc. 2, 4, 6, 8, 10µg/ml respectively.

2. For RP-HPLC [7-9]

Preparation of 0.1% Perchloric acid

In a 1000 ml measuring cylinder, take 700 ml of HPLC grade Water, add 1 ml of Perchloric acid and mix well, make up to the mark with HPLC grade water. Filter twice using 0.45µ nylon filter membrane and sonicate to degas for 15 min.

Standard Preparation

Initially Prepare a Standard Stock Solution (SSS-2) of Sodium Cromoglycate by adding 5mg in 10 ml volumetric flask & add 5 ml diluent (Acetonitrile : 0.1% Perchloric acid = 50 : 50) and mix, sonicate for 5 min. Make up the volume upto 10 ml with diluent, Conc. = 500 µg/ml is final Stock Solution.

Ex. Pipette out 1.0 ml of SSS-2 in 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent.(Conc. = 50 µg/ml).

RESULTS AND DISCUSSION

Method validation was done by using Validation parameters i.e. Linearity, Range, Accuracy, Precision, Specificity, System suitability, Robustness, Repeatability, Ruggedness etc. with the help of Spectrophotometry and Chromatographic methods as per ICH guidelines.

Stationary phase is, Agilent Zorbax SB-Aq (250 x 4.6mm, 5µ).

Diluents are, 50% 0.1% PCA : 50% ACN.

From table 1, 4th no. method was finalized for the chromatographic validation. In this trail, 1:1 conc. of PCA and ACN were used at 272nm wavelength, and obtained retention time (RT) of this method was 4.59 min, theoretical plates (TP) were 8876 and asymmetry factor was 1.11.

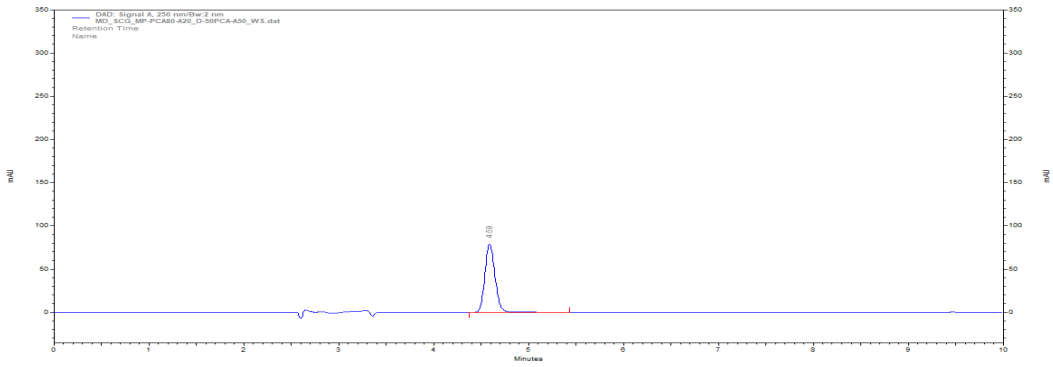

Fig. 2 : Optimized chromatogram of SCG

- ✓ **Chromatographic Conditions**:

Column Oven Temp	30°C
Flow Rate	1 ml/min.
Mobile Phase	0.1% Perchloric acid : Acetonitrile (80 : 20, % v/v)
Runtime	10 minutes
Injection Volume	10µl
Wavelength	272 nm
Diluent	Acetonitrile : 0.1% Perchloric acid (50 : 50, % v/v)
Column	Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)

Range

Range of an analytical procedure for spectroscopy is from 2 to 10 µg /ml & range for RP-HPLC is 40 to 60 µg/ml. Shown in table 2.

Selection of wavelength

The 10µg/ml standard solution was scanned in UV spectrophotometer from a range of 200 -400 nm against acetonitrile as blank & maximum absorption was found at 272nm shown in fig. 3

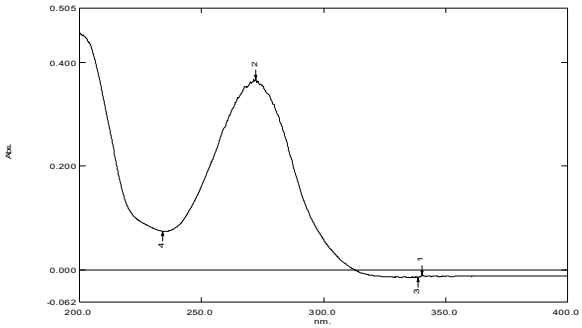

Fig. 3 : UV- Visible spectra of SCG

Linearity

The drug shows linearity in the range of 2 -10µg/ml for UV-Visible & & 40 -60µg/ml for Chromatography respectively. Shown in table 3, 4 respectively.

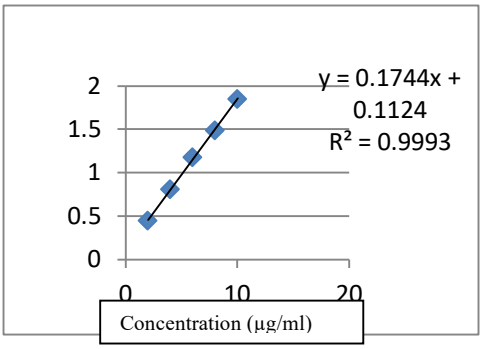

Fig. 4: Linearity curve for UV-Visible spec.

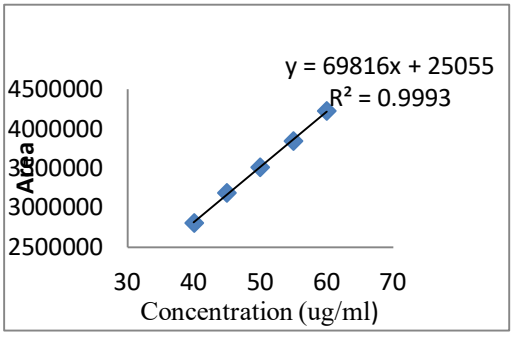

Fig 5 : Linearity curve for RP-HPLC

Limit of detection and Limit of quantitation (LOD, LOQ)

LOD = 3.3σ/$ Which is found as 2.52

LOQ = 10σ / $ Which is found as 7.64

Where, σ = standard deviation of the response

$ = slope of the calibration curve.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The mean absolute recovery of Sodium cromoglycate was from 92.83 to 102.16%. Here three levels was performed, the recovery of first level was 99.64%, for second level 99.75% and for third level 100.17%. The results were shown in table 6.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.Precision is divided into two class i.e. Intra-day precision and Inter-day precision which was studied in UV -Visible spectrophotometry and one another part of precision i.e repeatability which was studied in HPLC chromatographic technique [10-12]. The % RSD of UV spectroscopy is found to be less than 2 % and RP-HPLC (Repeatability) is 0.95% shown in table 7 and 8 respectively.

Robustness

Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 2% in the ratio of Acetonitrile in the mobile phase, varying flow rate ± 2ml, change in wavelength ± 2 for chromatography and UV Spectroscopy. Shown in table 9 & 10

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

There is no interference of the blank solution in region of the main peak of SCG . Hence the solvent system & chromatographic conditions are specific for the method.

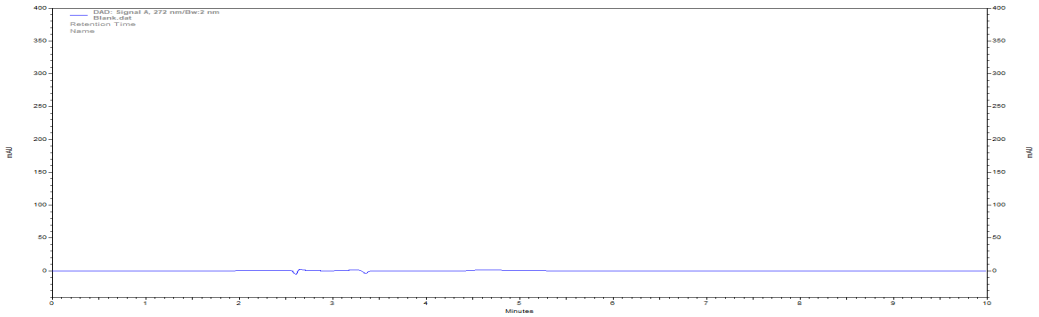

Fig. 6 : Chromatogram of blank solution

System suitability

The test is based on the concept that the equipment, electronics analytical operation and sample to be analyzed constitute an integral system that can be evaluated as such.

System suitability parameters are as below:

Retention time,

Theoretical plates,

Asymmetry (Tailing factor). Shown in table 11.

Assay

Assay was performed by using marketed ophthalmic solution of the SCG eye drop**.**

- Assay of SCG with the help of UV- Visible spectroscopy.

% purity = Absorbance of test solution / Absorbance of standard solution ×100

= 0.690 / 0.680×100

% purity = 101.47%

- Assay of SCG with the help of RP-HPLC.

% Assay = Sample area / Standard area ×100

= 3533358.83/ 3492674

% Assay = 98.85 %

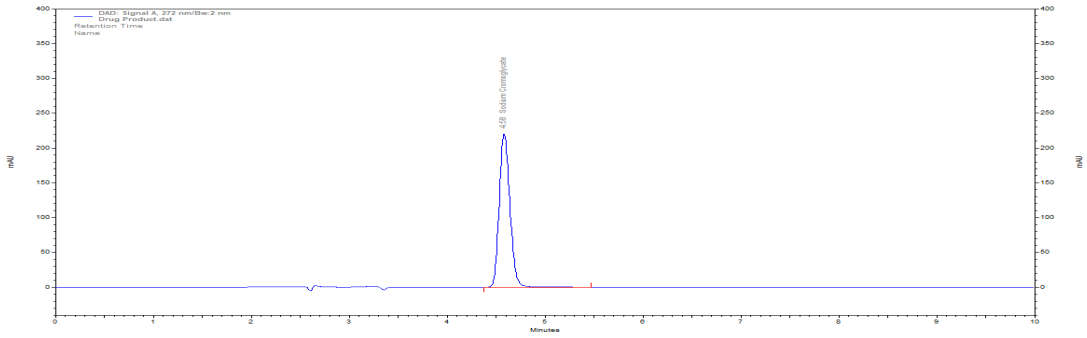

Fig. 7 : Chromatogram for marketed eye drop of SCG

Table 1 : Chromatographic Trials

Sr.No.	Mobile phase	λ Max	RT	TP	Asym.
1	0.1% Perchloric acid – Acetonitrile =50:50	250	2.92	554	0.73
2	0.1% Perchloric acid – Acetonitrile =60:40	250	2.95	1576	0.83
3	0.1% Perchloric acid – Acetonitrile =70:30	250	3.27	3469	0.77
4	0.1%Perchloric acid– Acetonitrile =80:20	272	4.59	8876	1.11
5	0.1% Perchloric acid – Acetonitrile = 90;:10	272	12.43	8992	1.10

Table 2: Conc. Ranges of UV-spectroscopy & RP-HPLC

Sr. No.	1	2	3	4	5
Conc.(µg/ml)					
UV- Spec.	2	4	6	8	10
RP-HPLC	40	45	50	55	60

Table 3 : Linearity data for UV- Visible spec.

Sr. No.	Conc.in (µg/ml)	Conc. in (%)	Absorbance
1	2	80	0.45
2	4	90	0.816
3	6	100	1.18
4	8	110	1.492
5	10	120	1.856

Table 4 ; Linearity data for RP-HPLC

Sr. No.	Conc. in (µg/ml)	Conc. in (%)	Area
1	40	80	2808897
2	45	90	3186270
3	50	100	3512722
4	55	110	3848126
5	60	120	4223381

Table 5. LOD, LOQ data for UV and RP-HPLC

Sr. No.	Parameters	UV Observation	HPLC Observation
1	Range (µg/ml)	2 -10	40 -60
2	Slope	0.1744	69816.48
3	Correlation coefficient (R^2)	0.9993	0.9993
4	LOD	0.05440	2.52
5	LOQ	0.1648	7.64
6	Intercept	0.112	25055

Table.6 : Accuracy data

Sample	Amt. Spiked (µg/ml)	Amount Recovered	% Recovery	AVG		%RSD	
				RP-HPLC	UV-Vis	RP-HPLC	UV- Vis
80%	39.88	39.63	99.37	99.64	92.83	0.34	1.876927
	39.88	39.69	99.52				
	39.88	39.89	100.03				
100%	49.85	49.56	99.42	99.75	102.1	0.32	0.941611
	49.85	49.88	100.05				
	49.85	49.74	99.79				
120%	59.82	59.59	99.61	100.1	97.77	0.49	1.31672
	59.82	60.10	100.46				
	59.82	60.09	100.45				

Table. 7. Repeatability data for Chromatography

Sr. No	Absorbance (INTRA-DAY)			Absorbance (INTER-DAY)		
Conc. (µg/ml)	2	6	10	2	6	10
1	0.445	1.18	1.85	0.45	1.18	1.856
2	0.452	1.22	1.76	0.454	1.2	1.81
3	0.45	1.19	1.79	0.465	1.84	1.805
4	0.453	1.23	1.80	0.459	1.18	1.83
5	0.448	1.22	1.82	0.46	1.23	1.85
6	0.45	1.18	1.84	0.457	1.19	1.84
Avg.	0.4496666	1.2046666	1.8125	0.4575	1.194	1.8318333
SD	0.0028751	0.0232780	0.03243300	0.00476445	0.0191833	0.0208846
%RSD	0.6394027	1.9323212	1.78940733	1.04141020	1.6066437	1.1400929

Table 8: Precision data for SCG

Sr. No	Conc. (µg/ml)	Area
1	40	3512722
2	40	3535254
3	40	3525785
4	40	3508674
5	40	3518977
6	40	3598741
Avg.		3533358.833
SD		33400.11779
%RSD		0.95

Table.9 : Change in wavelength 270nm & 274nm

Sr. No Conc. (µg/ml)	Absorbance at 270 nm			Absorbance at 274 nm		
	2	6	10	2	6	10
1	0.41	1.15	1.799	0.40	1.175	1.846
2	0.42	1.13	1.84	0.41	1.143	1.842
3	0.42	1.16	1.85	0.42	1.177	1.850
4	0.41	1.17	1.847	0.40	1.179	1.831
5	0.40	1.12	1.841	0.41	1.178	1.853
Average	0.41	1.146	1.83545	0.406	1.1704	1.844
SD	0.0070710	0.02073644	0.0207677	0.0054772	0.0154023	0.00856154
%RSD	1.7246506	1.80946259	1.1311511	1.3990703	1.315966	0.46419116

Table.10 : Change in flow rate 0.8ml, 1.0ml & 1.2 ml.

Conditions	Sample ID	Area	% Assay	RT	TP	Asymmetry
0.8ml	WS DP	3687814 3652145	- 99.03	4.62 4.62	8324 8317	1.08 1.11
1.0ml	WS DP	3533358. 3492674	- 98.85	4.58 4.58	8287 8178	19 1.15
1.2ml	WS DP	3692545 3659874	- 99.12	4.52 4.52	8237 8211	1.12 1.15

Table 11. system suitability data for RP-HPLC

Sample	RT	TP	Asymmetry
Rep 1	4.58	8287	1.19
Rep 2	4.58	8365	1.10
Rep 3	4.58	8214	1.12
Rep 4	4.58	8202	1.09
Rep 5	4.58	8352	1.08
Rep 6	4.58	8288	1.15
Average SD %RSD	4.58 9.72951E-16 000		

CONCLUSION

The present investigation represents a simple, accurate & cost effective UV-Visible & RP-HPLC method development study was done. The total run time was less than 10 min and retention time was 4.59min.with cost effective solvents.

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CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ETHICS STATEMENT

This study does not involve experiments on animals or human subjects.

REFERENCES

1. Paparaju.Sowjanya, (2013). Validated RP-HPLC method for estimation of sodium cromoglycate in human plasma. IJPRD. Vol 5(01): (112 – 120)
2. Ng LL, (1994). Reversed-phase liquid chromatographic determination of cromolyn sodium in drug substance and selected dosage forms. JAOAC 77(6), 1689-1694.
3. Mawatari K, Mashiko S, Sate Y, Usui Y, Iinuma F, Watanabe M,(1997). Determination of Disodium cromoglycate in Human Urine by High-performance Liquid Chromatography With Postcolumn Photo irradiation–Fluorescence Detection analyst,; 122(7), 715-717.
4. OzouxML,Girault J,Malgouyat JM,Pasquier O, (2001). Determination of sodium cromoglycate in human plasma by liquid chromatography–mass spectrometry in the turbo ion spray mode JChromatogr B Biomed Appl,; 765(2),179-185.
5. Sonia T. Hassib, Asmaa A. El-Zaherb, Marwa A. Fouad. (2011), Development and validation of RP-HPLC stability-Indicating methods for the determination of butamirate citrate and Sodium Cromoglycate. J. Chem. Pharm. Res., 3(6):243-258.
6. Paparaju.Sowjanya, Nissankararao.Srinath, Kamatham.Srinivas.Sumanth, Devarapalli.Chaitanya, Ammula.Pushpa Sai, Vantakula.Padmaja. (2013). Validated RP-HPLC method for estimation of sodium cromoglycate in human plasma. IJPRD, Vol 5(01): (112 – 120) .

7. Maha A. Hegazy , Medhat A. Al-Ghobashy, Basma M. Eltanany and Fatma I. Khattab.(2015). Validated chromatographic methods for the simultaneous determination of sodium cromoglycate and oxymetazoline hydrochloride in a combined dosage form. Journal of Advances in Chemistry Vol. 11, No. 8.
8. M. E. Fathy, S. Abo El Abass Mohamed, H. Elmansi, and F. Belal. (2017), Simultaneous determination of cromolyn sodium combined dosage forms using isocratic HPLC method. Journal of Chromatographic Science, Vol. 55, No. 1, 14–22.
9. El-Kosasy AM, Omar AA, Magdy N and El Zahar NM. (2016),Validated stability indicating HPLC method for determination of the polyanionic cromolyn sodiumi in presence of its alkaline degradate. Analytical Chemistry: An Indian Journal Vol-16, Iss – 13.
10. Maha A. Hegazy, May H. Abdelwahab, Hassan A. M. Hendawy, Soheir A.Weshahy & Samah S. Abbas. (2018). Validated HPTLC and HPLC methods for determination of fluorometholone and sodium cromoglycate in presence of their impurities and degradation products; application to kinetic study And on rabbit aqueous humor.Journal of Liquid Chromatography & Related Technologies ISSN: 1082-6076.
11. Mohammed Shahid Ali, Syed Rafiuddin, Dalya Abdullah Al-Jawi, Yasmin Al-Hetari, Mohsin Ul Haq Ghori, Aamer Roshanali Khatri. (2008). Stability-indicating assay of sodium cromoglicate in ophthalmic solution using mixed-mode hydrophilic Interaction chromatography. J. Sep. Sci., 31, 1645 – 1650.
12. Miranda Kay Holman, 1 Stacy D Brown, 2 Dorcas Frempong, 2 Ashana Puri, and Steven Dinh (2022). Development and Validation of a Liquid Chromatography-Mass Spectrometry Assay for Determination of Cromolyn Sodium in Skin Permeation Studies J Anal Methods Chem. 2022; 2022: 7437905. doi: 10.1155/2022/7437905

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