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COMMUNICATION

High Performance Liquid Chromatographic Determination of Sodium Cromoglycate

Adriana Segall, Fabiana Vitale, Rita Ricci, Gabriel Giancaspro, and María T. Pizzorno*

Cátedra de Control de Calidad de Medicamentos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina

ABSTRACT

A simple and precise liquid chromatographic method was developed for the estimation of sodium cromoglycate (SC) in pharmaceuticals. The drug was chromatographed on a reverse phase C18 column. The eluants were monitored at a wavelength of 325 nm utilizing a mixture (80:20) of solution A: 0.025 M 1-octane sulfonic acid sodium salt in acetic acid (1:100), and solution B: methanol, acetonitrile (3:2). Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for its linearity, accuracy, precision, selectivity, limit of detection and limit of quantitation. Due to its simplicity and accuracy, the authors believe that the method can be used for routine quality control analysis. It does not require any specific sample preparation except for the use of a column guard before the analytical column and a suitable prefilter attached to the syringe prior to injection.

INTRODUCTION

Sodium cromoglycate (disodium 5,5′-[(2-hydroxytrimethylene)dioxy]bis(4-oxo)-4H-1-benzopyran-2-carboxylic acid) (SC) which is also known as disodium cromoglycate and cromolyn sodium is an anti-allergic agent used prophylactically in disorders including asthma and rhinitis. It is not absorbed from the gastrointestinal tract and efforts have been made to find a similar substance which is absorbed when taken by mouth.

Methods for the determination of SC include spectrophotometry at 326 nm which is described in U.S.P. XXIII and a high-performance liquid chromatography

*To whom correspondence should be addressed.

(HPLC) on an anion-exchange column (1), a reversed phase for an ion-pair chromatography (2,3), and reversed phase HPLC with C8 and C18 using methanol-phosphate buffer as the mobile phase (4,5).

The purpose of this paper is to present a new method for SC analysis. Compared with HPLC on an anion-exchange column and different mobile phases including methanol, acetonitrile, and phosphoric or acetic acid, over reversed phase HPLC, the new method offers better tailing factor and resolution.

MATERIALS AND METHODS

Solvents and Chemicals

The authentic working standard for SC was developed locally using crystallizing technique. 1-Octane sulfonic acid, sodium salt was supplied by Sigma Chemical Co. St. Louis, USA (lot N° 124H5714). Solvents were of HPLC grade. Water HPLC grade was obtained by distillation and passed through a 0.45 micron membrane filter.

Apparatus

The HPLC system consisted of a dual piston reciprocating pump (Model KNK-500 G), a detector UV-Vis (Model KNK-029-757), an integrator (Model SP 4600) (all from KONIK) and a Rheodyne injector (Model 7125).

Chromatographic Conditions

The experiment was performed on a LiChroCART^R 250*4 mm HPLC Cartridge LiChrospher^R 100 RP-18 (5μm) Merck, coupled with a column guard of LiChroCART^R 4*4 mm LiChrosorb^R RP-18 (5 μm) Merck (Darmstadt, Germany). The mobile phase consisted of 80% solution A and 20% solution B. Solution A was 0.025 M 1-octane sulfonic acid sodium salt in acetic acid (1:100), and solution B was methanol, acetonitrile (3:2), degassed with helium. The flow rate was 1.00 mL/min and the run time 15 min. The column was used at room temperature. In these conditions SC retention time (t_R) was about 4 min. The wavelength was set at 325 nm. The volume of each injection was 50 μl.

Preparation of Sodium Cromoglycate Standard Solution

A stock standard solution of SC, 1 mg/ml, was prepared in water. The stock was suitably diluted with mobile phase to obtain a final concentration of 40 mcg/ml.

Preparation of Solutions Used for Assay Validation

For the study of SC response linearity, five solutions were prepared in mobile phase from SC stock standard solution at concentrations ranging from 20 mcg/ml to 60 mcg/ml.

System precision was evaluated by performing six consecutive injections of SC solution, one day apart and a week apart, by three different operators. Method precision was evaluated by six repeated assays of the same lot of one commercial formulation.

Procedure accuracy was evaluated by adding known amounts of the drug to SC stock solution and then diluting.

Method selectivity was determined from degrading SC under the following conditions:

a) 25 ml of SC stock standard solution was refluxed 1 hr.
b) SC was stored in an oven at 110°C for 24 hr.
c) SC was maintained in an open container exposed to daylight for 24 hr.
d) 25 ml of SC solution at 4 mg/ml concentration of in HCl 1N was refluxed 1 hr.
e) 25 ml of SC solution at 4 mg/ml concentration in NaOH 1N was refluxed 15 min.
f) 25 ml of SC solution at 4 mg/ml concentration in HCl 1N/Zn was refluxed 1 hr.
g) 25 ml of SC solution at 4 mg/ml concentration in H_2O_2 was refluxed 1 hr.

Procedure

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order to minimize solvent evaporation.

Prior to injecting solutions, the column should be equilibrated for at least 30 min with the mobile phase flowing through the system. Acceptable results for the number of theoretical plates, tailing factor, and precision, were calculated using equations U.S.P. XXIII, and detector linearity criteria were required before sample analysis. The volume of each injection was at least 50 μl.

Quantitation was accomplished using an external standard method. Every solution was injected in triplicate and the coefficient of variation (CV) was required to remain below 1.0% on an SC peak area basis.

RESULTS AND DISCUSSION

Initially, an anion-exchange column (1) was assayed, but a tailing factor (tf) lower than 2 could not be obtained, despite varying pH, buffer concentration and μg of sample injected. A reversed phase C18 columns was then assayed with mobile phases for acidic substances such as methanol, acetonitrile, or phosphoric acid mixtures but again tf proved greater than 2. On adding 1-octane sulfonic acid sodium salt at 10 mM concentration to one of the above mobile phases, a tf value of 1.8 was achieved, which became our definite eluant.

Figures 1 and 2 show the effect of 1-octane sulfonic acid sodium salt addition to the mobile phase on tf and t_R. Optimum conditions for solution A were thus 0.025 M 1-octane sulfonic acid sodium salt in acetic acid (1:100).

Linearity

Five solutions containing SC at concentrations ranging from 20 μg/ml to 60 μg/ml. were analyzed. The curve of peak areas versus concentrations (Y = 66.953X + 92) was linear with a coefficient of correlation r = 0.99955 and the intercept values were not significantly different from zero, (Figure 3).

Precision

CVs of assay system precision were 0.5%, 0.8%, and 0.7% at three different times while assay method precision was CV was 1.0%.

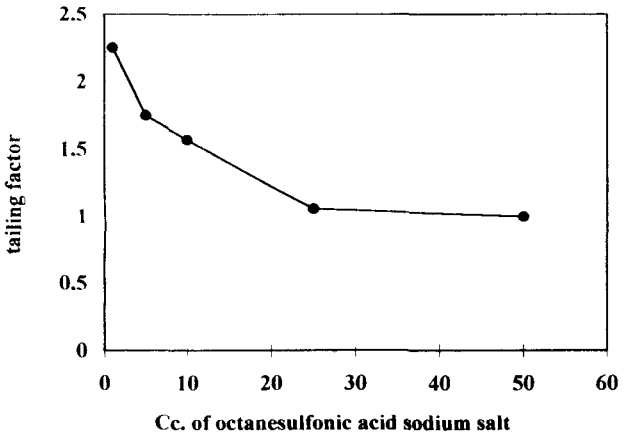

Figure 1. Sodium cromoglycate assay: effect of 1-octanesulfonic acid sodium salt addition to mobile phase on tailing factor.

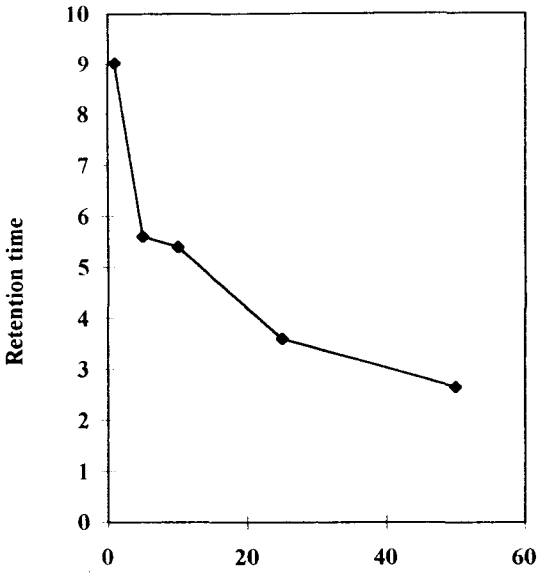

Figure 2. Sodium cromoglycate assay: effect of 1-octanesulfonic acid sodium salt on retention time.

Accuracy

Recovery data obtained from the study was in the range from 98.2 to 100.8% and relative standard deviation was 0.95%, (Table 1).

Selectivity

SC showed degradation products under: oxidation, alkaline hydrolysis, and reduction.

Selectivity was demonstrated showing that SC peak was free of interference from degradation products, (Figure 4).

Table 1

Sodium Cromoglycate Assay: Method Accuracy

Amount added (mg)	Amount Recovered (mg)	% Recovered
11.6	11.538	99.5
19.6	19.241	98.2
29.3	28.880	98.6
39.3	39.270	99.9
49.5	49.879	100.8
61.2	61.159	99.9

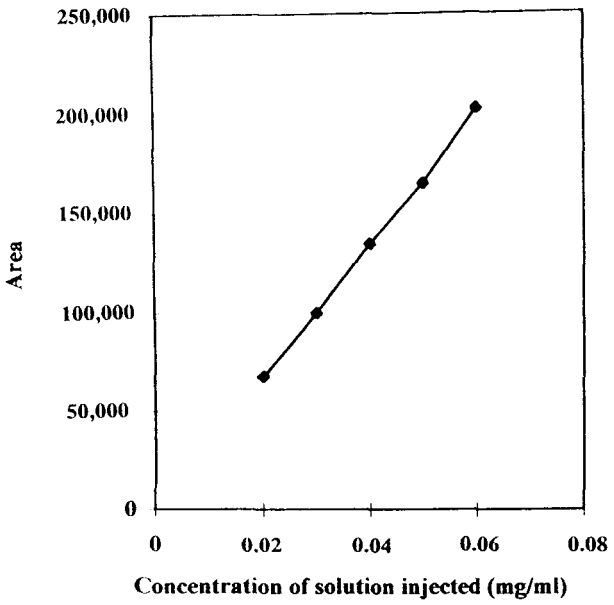

Figure 3. Sodium cromoglycate assay linearity.

Limit of Quantitation and Limit of Detection

The limit of detection (LOD) and the limit of quantitation (LOQ) of SC were calculated on peak areas, using the following equations:

$$LOD = 3 \times \frac{N}{B} \qquad LOQ = 10 \times \frac{N}{B}$$

where N, the noise estimate, is the standard deviation (SD) of peak areas of three solutions at SC concentrations of 0.6, 0.8, and 0.9 μg/ml; and B is the slope of the corresponding calibration curve. LOD was 0.06 μg injected and LOQ 0.19 μg injected.

CONCLUSIONS

The results of validation showed that the method was unaffected by assay time. SC and its degradation products were determined by the same chromatographic run and the analytical procedure described represents a selective, linear, precise and accurate method.

Given the simplicity of the proposed method, the authors suggest that it can be used in routine quality control analysis.

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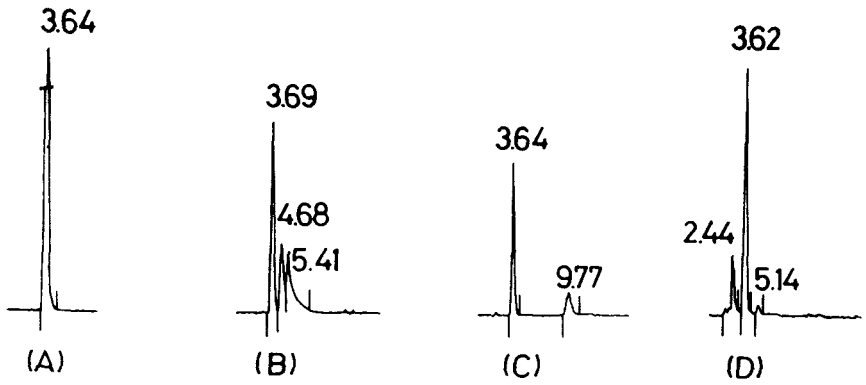

Figure 4. Chromatograms of sodium cromoglycate standard (A); alcaline degradation (B); reductive degradation (C); oxidative degradation (D).