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Determination of sodium cromoglycate in human plasma by liquid chromatography–mass spectrometry in the turbo ion spray mode

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Abstract

A highly sensitivity liquid chromatography–tandem mass spectrometry method has been developed for the quantitation of sodium cromoglycate (SCG) in human plasma. The method was validated over a linear range of 0.100–50.0 ng/ml, using $^{13}C_4$ sodium cromoglycate as the internal standard. Compounds were extracted from 1.0 ml of lithium heparin plasma by methanol elution of C_{18} solid-phase extraction cartridges. The dried residue was reconstituted with 100 μl of 0.01 N HCl, and 30 μl was injected onto the LC–MS–MS system. Chromatographic separation was achieved on a C8 (3.5 μm) column with an isocratic mobile phase of methanol–water–0.5 *M* ammonium acetate (35:64.8:0.2, v/v/v). The analytes were detected with a PE Sciex API 3000 mass spectrometer using turbo ion spray with positive ionization. Ions monitored in the multiple reaction monitoring (MRM) mode were *m/z* 469.2 (precursor ion) to *m/z* 245.1 (product ion) for SCG and *m/z* 473.2 (precursor ion) to *m/z* 247.1 (product ion) for $^{13}C_4$ SCG (I.S.). The average recoveries of SCG and the I.S. from human plasma were 91 and 87%, respectively. The low limit of quantitation was 0.100 ng/ml. Results from a 4-day validation study demonstrated excellent precision (C.V.% values were between 1.9 and 6.5%) and accuracy (−5.4 to −1.2%) across the calibration range of 0.100–50.0 ng/ml.

Keywords: Sodium cromoglycate

1. Introduction

Sodium cromoglycate, 5,5′-[(2-hydroxy-1,3-propanediyl)bis(oxy)]bis(4-oxo)-4*H*-1-benzopyran-2-carboxylic acid disodium salt, is an effective and commonly used treatment for asthma. Early studies using ^{14}C-labelled compound showed that it was rapidly absorbed and that the drug was quickly excreted in bile and urine [1]. A reverse phase ion-pair liquid chromatographic method [2] and an HPLC assay using an anion-exchange column [3] were developed and validated for the quantitation of SCG in human urine. HPLC methods have also been described for the determination of SCG in dosage forms [4,5]. However, due to the low concentrations and the rapid clearance of SCG in human plasma, the most sensitive method has been a radio immunoassay [6] with a low limit of quantitation (LOQ) of 0.500 ng/ml. The first plasma pharmacokinetics for SCG in humans were based on concentration data obtained

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by this RIA method [7,8]. After inhalation of SCG (20 mg), a mean peak concentration of 46±7 ng/ml was reached at 5 min. Plasma concentrations thereafter declined extremely rapidly and were not detectable after 6 h. Some interference around the LOQ could occur in pre-dose samples with this radio immunoassay. Therefore, due to the compound's specific pharmacokinetic properties and questions regarding the specificity of the RIA method, it was necessary to develop and validate a highly sensitive and specific bioanalytical method to quantify SCG in human plasma which is reported herein.

2. Experimental

2.1. Reagents

Sodium cromoglycate (purity 99.7%) and $^{13}C_4$ sodium cromoglycate (internal standard, I.S.) were provided by Aventis Pharma (Fig. 1). The water content of SCG was determined prior to the preparation of aqueous standards using Karl-Fischer titration to prepare accurate standard solutions. Control (blank) lithium heparin plasma was provided by Parexel-Cemaf and was tested for any interference before use. Water and methanol (HiperSolv) were purchased from BDH and were of HPLC grade. Ammonium acetate for analysis and 2 *M* hydrochloric acid were obtained from Merck.

2.2. Materials

Bond Elut cartridges (C_{18}, 100 mg) for sample extraction were obtained from Varian (Merck Eurolab, Pessac, France). A Jouan centrifuge model GR412 (Jouan, Saint Herblin, France) and a Turbovap evaporator (Zymark, Roissy, France) were used. The mass spectrometer used was a Perkin-Elmer Sciex API3000 (Applied Biosystems, Toronto, Canada) and liquid chromatography was performed using a HP1050 system (pump plus autosampler) from Hewlett-Packard (Agilent, Orsay, France) and a C8 Symmetry HPLC column (3.5 μM, 50×4.6 mm I.D.) obtained from Waters (Saint Quentin en Yvelines, France).

2.3. Preparation of stock solutions

Primary stock solutions of SCG were prepared from separate weightings for standards and quality controls (QCs). The primary stock solutions were prepared in water and stored at −20°C during the validation. Stock solutions of SCG were prepared by dissolving the reference compound in water to obtain a solution at 100 μg/ml (expressed as free base and taking into account the solvent and water content). An I.S. stock solution was prepared in water at 1.00 mg/ml and stored at −20°C. A working solution at 0.400 μg/ml of I.S. was prepared by appropriate dilution of the stock solution in water and stored at +4°C. Working solutions of SCG were prepared in HPLC water, by appropriate dilution, at 1000, 800, 400, 200, 100, 40.0, 10.0, 4.00 and 2.00 ng/ml. These working solutions were stored at +4°C until used.

2.4. Preparation of standards

The standards were prepared daily in blank human plasma by the addition of 50.0 μl of one of the above working solutions (or water for the zero

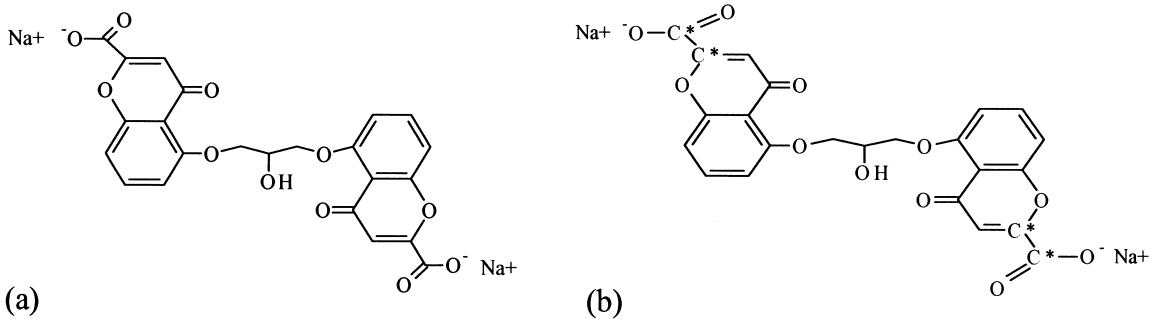

Fig. 1. (a) SCG; (b) I.S. *Indicates labelled carbons.

standard) to 1.0 ml of plasma for standard concentrations of 0.100–50.0 ng/ml.

2.5. Preparation of QCs

QCs were prepared daily in blank human plasma by the addition of 50.0 μl of a working solution to 1.0 ml of plasma. They were prepared at the minimum quantitation limit (MQL), low level, middle level and at the upper quantitation limit (UQL): 0.100, 0.150, 5.00 and 50.0 ng/ml, to determine the precision and accuracy of the assay and to evaluate the stability of the compound after three cycles of thaw/freeze.

2.6. Sample preparation

A solid-phase extraction method was developed to isolate SCG and the I.S. from human plasma. The 1.00-ml unknown plasma samples were spiked with 50 μl of I.S. and 50 μl of water. The standards and QCs were spiked with 50 μl of I.S. except the blank which received 50 μl of water. All the samples were transferred into 5-ml disposable glass tubes with 1.00 ml of water. The extraction cartridges were conditioned with 2 ml of methanol followed by 1 ml of water. Each diluted plasma sample was vortex-mixed for 10 s and applied to a cartridge. After washing with 1 ml of 0.1 *M* ammonium acetate and 1 ml of methanol–0.1 *M* ammonium acetate (15:85, v/v), the cartridge was placed over a 10-ml disposable glass tube. SCG and I.S. were eluted from the cartridge under atmospheric pressure using 1 ml of methanol. After evaporation to dryness under a gentle stream of nitrogen at +50°C, the residue was reconstituted into 100 μl of 0.01 *M* HCl, and 30.0 μl was injected into the LC–MS–MS system.

2.7. Chromatographic systems

Separation of SCG and I.S. was achieved on a HP1050 system using a 50×4.6 mm I.D., 3.5 μm particle size analytical column eluted with a methanol–water–0.5 *M* ammonium acetate mobile phase (35:64.8:0.2, v/v/v). The HPLC system was operated isocratically at a flow-rate of 0.15 ml/min. The mobile phase was filtered on a 0.45-μm Sartolon® filter before use.

PE Sciex API 3000 mass spectrometer was operated using turbo ion spray in positive ionization mode with the following parameters: nebulizer gas at a pressure equal to 4 bar, turbo ion spray gas (nitrogen) at 6 l/min, and the curtain gas (nitrogen) and exhaust (air) at a pressure of 6 bar. The temperature of the turbo ion spray source was set at +300°C.

Quantitative determination of the unchanged drug was performed by using the instrument in the multiple reaction monitoring (MRM) mode, in order to measure the following ion transitions: m/z 469.2 (m+H+) to m/z 245.1 (product ion) for SCG and m/z 473.2 (m+H+) to m/z 247.1 (product ion) for $^{13}C_4$ SCG (I.S.). The quasi molecular ions are present in the mass spectra as base peaks. Nitrogen was used as collision gas (30 eV) and the electron multiplier was set at 2200 V. After acquisition, the response signals were analysed using the Corvette® version 18-IV-1999 software, developed by Parexel-Cemaf.

2.8. Data treatment

Standard calibration curves were plotted as the chromatographic peak area ratio (SCG/I.S.) versus the corresponding nominal plasma SCG concentrations (0.100–50.0 ng/ml). A 1/concentration^2 weighted regression analysis was used to determine the slope, intercept and coefficient of determination (r^2) using Corvette®.

2.9. Validation procedure

During validation of the method, the following parameters were evaluated.

Specificity: During validation, six batches of blank lithium heparin human plasma were screened on the LC–MS–MS.

The extraction recovery of sodium cromoglycate was calculated by comparing the peak area ratio (SCG/I.S.) measured for human plasma spiked prior to being taken through the sample preparation procedure, with the peak area ratio obtained for control plasma extracts spiked after the extraction procedure, at the same concentration of SCG. The control plasma extracts were blank plasma spiked only at the end of the sample processing. The recovery was

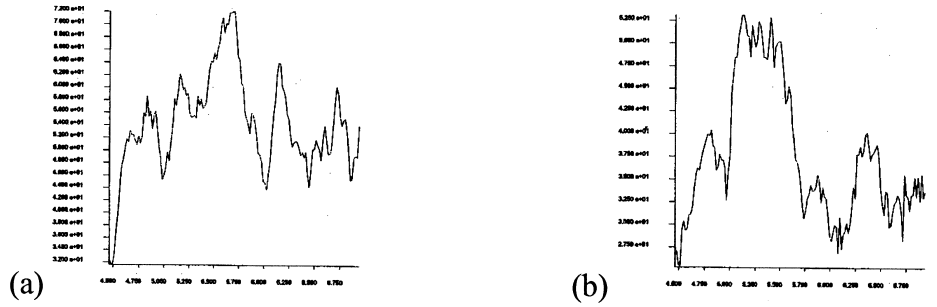

Fig. 2. Typical chromatogram obtained from a blank plasma sample. (a) I.S. transition; (b) SCG transition.

calculated at three concentration levels: 0.150, 5.00 and 50.0 ng/ml of SCG.

Range of standard calibration curve: The range of reliable ratio was determined during the performance of four analytical runs.

Minimum quantitation limit (MQL): The MQL was defined as the lowest concentration of SCG that could be measured with acceptable precision and accuracy (<15%), determined on six replicates of QC samples at 0.100 ng/ml assayed on four different occasions.

The precision and accuracy (defined as the [mean back-calculated concentration−nominal concentration]/nominal concentration×100) of the method were determined from the back-calculated concentrations of QC samples during a single run (six replicates of each level of QCs) and between runs (six replicates of each QC assayed on four different occasions).

The stability of SCG in human plasma after three cycles of freeze/thaw was investigated on a range of QCs (0.150, 5.00 and 50.0 ng/ml). The stability in the reconstituted plasma extracts was measured by analysing the same standards after a 12-day period storage at +4°C.

3. Results

3.1. Separation and specificity

No interfering peaks at the retention times of the compound of interest (5.7 min) were found on analysis from the six batches of plasma. Fig. 2 shows a typical chromatogram obtained from blank plasma. Figs. 3 and 4 show extracted control plasma spiked with 10.0 ng/ml of SCG or with 20.0 ng/ml of I.S., respectively.

All the chromatograms were gaussian in shape and

Fig. 3. Typical chromatogram obtained from a blank plasma sample spiked with 10 ng/ml of SCG. (a) I.S.; (b) SCG.

Fig. 4. Typical chromatogram obtained from a blank plasma sample spiked with 20 ng/ml of $^{13}C_4$ SCG. (a) I.S.; (b) SCG.

the base peak-width of each compound was less than 60 s.

3.2. Recovery

The results presented in Table 1 demonstrate that the recovery of SCG ranged from 96.4% at 0.150 ng/ml to 86.4% at 50.0 ng/ml. The overall recovery of I.S., calculated by control plasma spiked with 20.0 ng/ml of I.S. and 50.0 ng/ml of SCG, was ~87%.

3.3. Linearity and limit of quantitation

Standard calibration curves provided a reliable response from 0.100 to 50.0 ng/ml. The slopes were between 0.06309 and 0.06472, with intercepts between 0.00008 and 0.00126. The coefficients of determination were between 0.99835 and 0.99975. The results of the back-calculated concentrations of these standards showed a very low dispersion of the standards all along the calibration plot. The accuracy was between −1.7 and 1.0%.

3.4. Precision and accuracy

The within-run precision of the method, summarised in Table 2, was less than 4.3% and the accuracy was within −3.7% of the expected concentration.

The between-run precision of the method was less than 6.5% and the accuracy was within −5.4%, as shown in Table 3.

Between-run C.V.% of the calibration standards

Table 1
Recovery of SCG and I.S.

Sodium cromoglycate	0.150 ng/ml SCG		5.00 ng/ml SCG		50.0 ng/ml SCG		20.0 ng/ml I.S.	
Peak area ratio	Plasma extract	Non-processed standard	Plasma extract	Non-processed standard	Plasma extract	Non-processed standard	Plasma extract	Non-processed standard
	0.00953	0.00994	0.32040	0.34414	3.08680	No value	3.12109	3.54062
	0.00902	0.00942	0.30948	0.34799	3.09698	3.55526	3.23119	3.59787
	0.00908	0.00959	0.31344	0.35022	3.07285	3.53557	3.19042	3.61139
	0.00935	0.00983	0.31238	0.34756	3.03952	3.50618	3.20127	3.66440
	0.00964	0.01018	0.31108	0.35192	3.06002	3.51203	3.21459	3.73648
	0.00964	0.00940	0.31139	0.35216	3.06275	3.64776	3.21145	3.77770
Mean	0.00938	0.00973	0.31303	0.34900	3.06982	3.55136	3.19500	3.65474
SD	0.00028	0.00031	0.00385	0.00306	0.02049	0.05733	0.03870	0.08948
C.V.%	2.9	3.2	1.2	0.88	0.67	1.6	1.2	2.5
Overall recovery %		96.4		89.7		86.4		87.4

Peak area ratio of SCG/I.S. Sample spiked before extraction process/sample spiked after the extraction process.

Table 2
Within-run precision and accuracy of quality controls

Nominal concentration (ng/ml)	*n*	Mean (ng/ml)	SD	C.V. (%)	Accuracy (%)
0.1	6	0.103	0.004	4.3	3.4
0.15	6	0.150	0.007	4.3	0.14
5	6	4.82	0.11	2.4	−3.7
50	6	50.1	0.9	1.9	0.16

Table 3
Between-run precision and accuracy of quality controls

Nominal concentration (ng/ml)	*n*	Mean (ng/ml)	SD	C.V. (%)	Accuracy (%)
0.1	24	0.099	0.005	5.5	−1.2
0.15	24	0.147	0.010	6.5	−2.2
5	24	4.73	0.10	2.1	−5.4
50	24	49.4	0.9	1.9	−1.2

ranged from 0.25 to 2.2% and the accuracy was between −3.5 and 1.8%.

3.5. Minimum quantitation limit (MQL)

Fig. 5 shows the chromatogram of an extracted QC at the MQL.

The between-run precision of the 0.100-ng/ml QC samples was equal to 5.5% and the between-run accuracy was equal to −1.2%, demonstrating that this level was validated as the MQL for this assay.

3.6. Stability

To mimic the possible freezing and thawing conditions of clinical samples, QCs were subjected to multiple cycles of freezing and thawing and then analysed. After three cycles, the back-calculated concentrations of QCs were comparable to the concentrations of freshly prepared QCs (Table 4).

Additionally, the compound was found to be stable in reconstituted plasma extracts for 12 days at +4°C. The differences between the standards stored

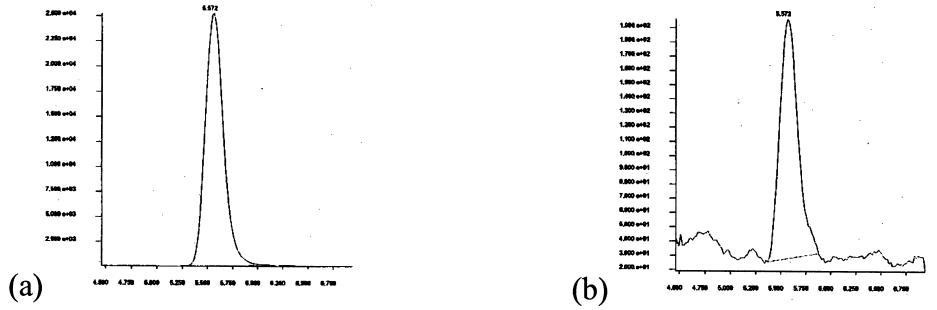

Fig. 5. Chromatogram of the quality control at the MQL. (a) 20.0 ng/ml of I.S.; (b) 0.100 ng/ml of SCG.

Table 4
Stability after three cycles of freeze/thaw at −20°C

Fresh reference (ng/ml)	*n*	After three cycles, mean (ng/ml)	SD	C.V. (%)	% Accuracy vs. reference
0.138	6	0.144	0.003	2.0	4.0
4.77	6	4.68	0.06	1.4	−1.9
50.7	6	49.0	0.4	0.89	−3.3

at +4°C versus the fresh reference was between −3.0 and 4.7%.

These results show that no significant degradation occurred during chromatography, extraction and sample processes.

4. Conclusions

A method using LC–MS–MS turbo ion spray with positive ionisation has been developed and validated for the quantitative determination of sodium cromoglycate in human plasma. The method is sensitive, specific, precise and accurate in the concentration range of 0.100–50.0 ng/ml of SCG. In comparison with the radioimmunoassay previously developed, it is easy to handle (no antibody to produce and no use of ^{125}I). The sensitivity and the specificity of this assay make it suitable for clinical pharmacokinetic studies of sodium cromoglycate.

References

[1] M.J. Ashton, B. Clark, K.M. Jones, G.F. Moss, M.G. Neale, J.T. Ritchie, Toxicol. Appl. Pharmacol. 26 (1973) 319.
[2] O.A. Aswania, S.A. Corlett, H. Chrystyn, J. Chromatogr. B 690 (1997) 373.
[3] J.J. Gardner, J. Chromatogr. 305 (1984) 228.
[4] D. Radulovic, V. Kosic-Pesic, D. Pecanac, L. Zivanovic, Farmaco 49 (1994) 375.
[5] L. Linda, J. AOAC Int. 77 (1994) 1689.
[6] K. Brown, J.J. Gardner, W.J. Lockley, J.R. Preston, D.J. Wilkinsin, Ann. Clin. Biochem. 20 (1983) 31.
[7] R.W. Fuller, J.G. Collier, J. Pharm. Pharmacol. 35 (1983) 289.
[8] M.G. Neale, K. Brown, R.W. Hodder, R.M. Auty, Br. J. Clin. Pharmacol. 22 (1986) 373.