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Short communication

Development and validation of an ion-pair liquid chromatographic method for the quantitation of sodium cromoglycate in urine following inhalation

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Abstract

An ion-pair liquid high-performance chromatography method with solid-phase extraction for measuring urinary concentrations of sodium cromoglycate following inhalation has been developed and validated. Sodium cromoglycate was extracted from urine on a 100-mg phenyl cartridge (Isolute, Jones Chromatography) and then quantified on a 25-cm C_8 Spherisorb 5 μm stationary phase with a mobile phase of methanol–0.045 *M* phosphate buffer–0.05 *M* dodecyl triethyl ammonium phosphate (550:447.6:2.4, v/v) pH 2.3, at 0.85 ml min^{-1} using nedocromil sodium as an internal standard and UV detection at 238 nm. The inter- and intra-day reproducibilities were 8.33 and 13.63%, respectively, at 0.25 mg l^{-1}. The limit of determination for sodium cromoglycate was 0.25 mg l^{-1} (with a signal-to-noise ratio of greater than 10:1). Following oral and inhaled administration of 20 mg of sodium cromoglycate to eight healthy volunteers, the mean and S.D. of sodium cromoglycate excreted in the urine at 0.5, 1 and 24 h post-dose were 0.02, 0.05 and 0.33%, and 0.16, 0.30 and 1.55% of the dose, respectively. The urinary recovery of sodium cromoglycate at 0.5 and 1 h following inhalation can therefore be used to compare the amount of drug reaching the respiratory tract using different sodium cromoglycate inhaled products or inhalation methods.

Keywords: Sodium cromoglycate

1. Introduction

Sodium cromoglycate (SCG) is considered to be the safest therapeutic agent in current use for the prophylactic management of both extrinsic and intrinsic bronchial asthma [1]. Clinical studies have shown that treatment with SCG is always beneficial in mild to moderate exercise-, cold air- and allergen-induced asthma; and in children with cough variant asthma [2].

SCG is poorly absorbed from the gastrointestinal tract [3], but is completely absorbed from the lung [4]. It is not metabolised. SCG, delivered to the body is cleared rapidly from the plasma and is excreted in the bile and urine in approximately equal proportions [5]. The amount of SCG absorbed after inhalation varies according to the method of inhalation and the dose administered [5]. After inhalation of 20 mg SCG via a Spinhaler (Fisons, Loughborough, UK)

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approximately 4% of the nominal dose is absorbed, 2% is excreted in the urine and 84% is recovered from the faeces. Once absorbed SCG appears in the urine within a few minutes due to its rapid renal elimination [5]. We have previously reported a method to compare the relative lung bioavailability of salbutamol to the lungs following the inhalation of different products and using different inhalation methods [6]. If a quick and cheap method to assay SCG in urine was available, the same principles as those used for salbutamol [6] could be used to compare relative lung bioavailability of SCG.

Analytical methods for the determination of SCG in aqueous solutions, pharmaceutical dosage forms and in biological fluids have been reported; using various techniques including spectrophotometry [7], fluorimetry [3] and polarography [8]. Titration of SCG in non-aqueous media has also been used [9]. However, these methods are either not suitable for the measurement of SCG in biological fluids [7–9] or are time consuming and complicated procedures [3] which are not suitable for routine clinical studies.

A reversed-phase high-performance liquid chromatography (HPLC) assay has been reported by Radulovic et al. [10] for the determination of SCG in pharmaceutical dosage forms. An ion-exchange HPLC method to measure SCG in urine samples has been reported by Gardner [11]. Although this latter method is reported as suitable for clinical trials, it uses strong buffer solutions at high flow-rates and therefore, reproducibility of the analytical conditions are difficult to achieve. SCG concentrations in plasma have been measured using radioimmunoassay [12]. Although this method is sensitive and specific for SCG, it requires, expensive reagents and a complicated handling procedure. Vidgren et al. [13] have developed a method to measure the amount of SCG deposited in the lungs after inhalation using ^{99m}Tc-radiolabelled SCG particles. SCG is co-precipitated with ^{99m}Tc using a spray drying technique. However, applications of this method are limited due to a reduction in radioactivity of 38% after only 10 min post inhalation.

Accordingly, the aim of our work was to develop a sensitive, rapid and easy to perform method for measuring urinary concentrations of SCG following an inhaled dose in volunteers.

SCG was isolated from urine using solid-phase extraction (SPE), and then quantified on a C_8 Spherisorb stationary phase with a mobile phase containing a long chain alkyl triethyl ammonium phosphate as an ion-pairing agent. Nedocromil sodium (NCS) was used as internal standard and UV detection at 238 nm was employed. This method permits the detection of SCG in human urine at concentrations as low as 0.2 mg l^{-1} with acceptable accuracy and precision and may be used to determine SCG in urine samples from volunteers collected up to 24 h post-inhalation.

2. Experimental

2.1. Chemicals

Intal 5-mg metered dose inhaler, sodium cromoglycate and nedocromil sodium bulk drug are products of Fisons. Dodecyl triethyl ammonium phosphate (HPLC grade) was purchased from Regis (Skokia, IL, USA). Methanol, sodium dihydrogenphosphate, orthophosphoric acid, sodium hydroxide and hydrochloric acid were purchased from BDH (Poole, UK) and were of HPLC or analytical grade.

The water content of SCG was determined immediately prior to the preparation of aqueous standards using Karl–Fischer titration so that accurate standard solutions could be used.

Highly purified double distilled water was used throughout the study.

2.2. Sample preparation

A solid-phase extraction method using phenyl (PH) cartridges to isolate SCG and its internal standard (NCS) from urine samples has been developed. A phenyl cartridge was selected from the range of non-polar solid-phases which were tested, because it gave the most efficient sample clean up with the highest recovery of SCG and internal standard.

Prior to extraction, 1 ml of aqueous standard containing SCG and NCS (20 mg l^{-1}) was added to 5 ml blank urine and 4 ml 0.25 *M* hydrochloric acid to produce standard urine concentrations of 0.25–2 mg l^{-1} SCG, pH 1. Pooled (24 h), blank urine samples were obtained from seven volunteers (three

of whom were female). For clinical samples 1 ml of aqueous standard solution containing 20 mg l^{-1} NCS was added to 5 ml of urine and 4 ml of 0.25 *M* hydrochloric acid. Hydrochloric acid (0.25 *M*) was added to acidify the sample and thus to facilitate its extraction onto a non-polar cartridge. After vortex-mixing for 30 s, 2-ml aliquots were removed for extraction.

PH cartridges (100 mg, Isolute, International Sorbent Technology, UK) were inserted into the lid of a Vac Elut station (Jones Chromatography, Mid Glamorgan, UK). Up to ten cartridges could be extracted at one time.

The cartridges were conditioned with 2 ml methanol followed by 2 ml 0.1 *M* hydrochloric acid. Great care was taken to ensure that the meniscus of the conditioning solution was kept above the top of the sorbent bed prior to the addition of acidified urine to prevent air being drawn through the columns. Following conditioning 2 ml of the acidified dilute sample (standard or clinical) was applied to the cartridge and allowed to elute through the bed over 2 to 3 min. After applying a full vacuum for 3 min, the columns were washed with 2 ml of 0.1 *M* hydrochloric acid, followed by 2 ml 30% methanol in 0.1 *M* hydrochloric acid, and then dried by applying a full vacuum for 3 min. The analytes (SCG and NCS) were eluted with 2 ml methanol.

After evaporating to dryness under a stream of nitrogen the residue was reconstituted in 1 ml of mobile phase. The extract coating the vial walls was dissolved by vortex-mixing for 30 s prior to injecting the samples onto the HPLC system.

2.3. HPLC equipment and analytical method

The HPLC system consisted of a Gilson Model 307 pump (Anachem, Luton, UK) and an Applied Biosystems Model 795 A ultraviolet detector (Anachem), set at 238 nm. The detector was linked to a Shimadzu CR-6A integrator (Dyson Instruments, Haughton-le-Spring, UK). Aliquots (20-μl) were injected using a Shimadzu SIL-9A autosampler (Dyson Instruments).

Spherisorb S5C8, 25 cm×4.6 mm I.D. and 1 cm×4.6 mm I.D. columns were used as the stationary and guard column, respectively (Hichrom, Reading, UK).

The mobile phase was methanol–0.045 *M* phosphate buffer–0.5 *M* dodecyl triethyl ammonium phosphate (550:447.6:2.4, v/v) adjusted to pH 2.3 by the addition of orthophosphoric acid. The mobile phase was filtered using 0.45-μm membrane filters (Millipore) and degassed in an ultrasonic bath under vacuum for 30 min prior to use. A constant flow-rate of 0.85 ml min^{-1} was used to deliver the mobile phase through the system and ambient temperature was employed throughout the study.

2.4. Volunteer study

Eight (four female) healthy volunteers with no history of asthma gave written informed consent to enter the study. Local ethical committee approval was obtained. Volunteers were well trained in inhalation technique before inclusion. On study days, volunteers either inhaled four puffs of Intal 5 mg or swallowed 20 mg SCG powder dissolved in 25 ml of water. The study was randomised and crossed-over after 1 week.

Urine was collected at 0, 0.5, 1, 2, 3, 5, 8, 12 and 24 h post-dose. All samples were frozen at −20°C prior to analysis.

3. Results and discussion

Chromatograms resulting from the analysis of a blank urine sample, a standard urine sample containing 1 mg l^{-1} SCG and 2 mg l^{-1} NCS, and a volunteer sample 0–30 min post-inhalation are shown in Fig. 1a–c, respectively. SCG and its internal standard were eluted from the column with capacity factors (k) 8.5 and 10.8, respectively. These correspond to retention times of 19.0 and 23.5 min. The retention times of SCG and NCS increased with increasing concentrations of the ion-pair reagent and decreased with increasing concentrations of the organic modifier. The pH of the mobile phase also affected the retention times and the peak separation and therefore all were carefully controlled.

Evaluation of the assay was carried out using a five-point calibration over the range 0.25–2.0 mg l^{-1} SCG in urine. A calibration curve was obtained by plotting peak-height ratio of the SCG to the internal standard, against the nominal SCG concentration. A

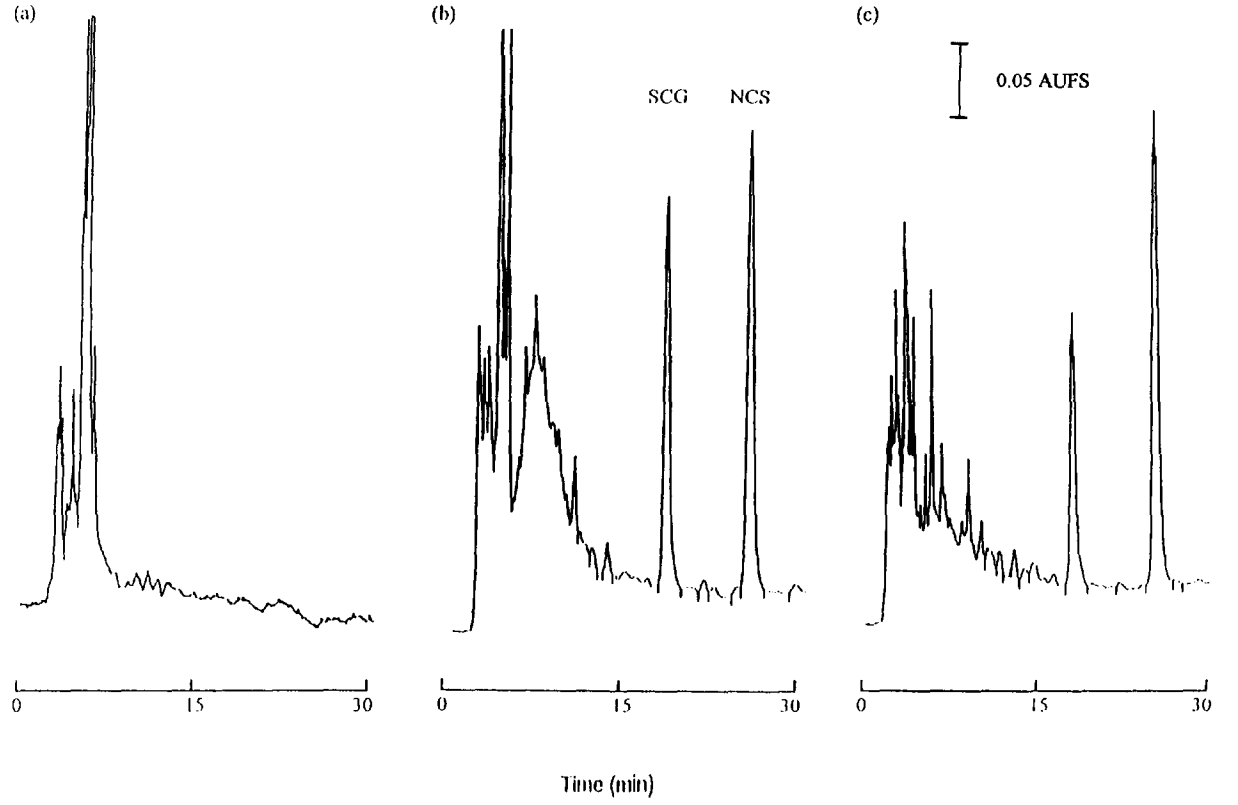

Fig. 1. Chromatogram of (a) blank urine sample, (b) standard urine sample containing 1 mg l^{-1} SCG and 2 mg l^{-1} NCS, and (c) volunteer sample 0–30 min post-inhalation.

straight line was fitted to the data by linear regression. The calibration curves were linear over the concentration range investigated with regression coefficients of greater than 0.9996.

The mean (S.D.) absolute recovery of SCG from urine samples containing 0.25, 0.5, 1.0, 1.5 and 2.0 mg l^{-1} (n=4) was 98.2 (0.04), 97.7 (0.13), 99.4 (0.09), 94.0 (0.13) and 84.7 (0.04)%, respectively.

The data presented in Table 1 demonstrates the precision and accuracy of this assay. Intra-assay variability was determined at the five standard urine concentrations of SCG in quadruplicate. Inter-assay variability was determined at the same five concentrations in four replicate runs on different days. The precision of the method (mean percentage coefficient of variation) and the accuracy (difference between nominal and found concentrations) for the values of recovered determined standards, when calculated as unknowns against the linear regression line, were acceptable (less than 15% variation from the nominal concentration) over the concentration range investigated.

The limit of determination, calculated, with a signal-to-noise ratio of greater than 10:1, from the mean and S.D. of the intercept of four calibration curves, was 0.25 mg l^{-1}. The limit of detection, calculated with a signal-to-noise ratio of 3:1, was 0.05 mg l^{-1}.

SCG appeared rapidly in the urine following inhaled administration. The rates of urinary excretion of SCG during the 24-h collection period post inhaled and oral dosing are shown in Fig. 2. The mean (S.D.) rate of SCG urinary excretion decreased from 63.6 (30.9) μg h^{-1} (0–30 min) to 5.78 (7.04) μg h^{-1} (12–24 h) post inhalation. In contrast, with the oral dose, the mean (S.D.) SCG urinary excretion rate increased gradually, reaching a maximum of 19.2 (8.0) μg h^{-1} 1–2 h after administration. The

Table 1
Method precision and accuracy

Concentration added (mg/l)	Concentration found (Mean±S.D., $n=4$) (mg/l)	Coefficient of variation (%)	Difference between nominal and found concentrations (%)
Intra-assay variation[a]			
0.25	0.24±0.02	8.33	4.0
0.50	0.53±0.02	3.77	6.0
1.00	0.99±0.01	1.01	1.0
1.50	1.52±0.12	7.89	1.3
2.00	1.98±0.06	3.03	1.0
Inter-assay variation			
0.25	0.22±0.03	13.63	12.0
0.50	0.53±0.02	3.77	6.0
1.00	1.00±0.10	10.00	0.0
1.50	1.51±0.08	5.29	0.6
2.00	1.97±0.17	8.62	1.5

[a] Regression equation for the intra-assay was $(y)=1.0715(x)+0.088793$.

mean (S.D.) rate of excretion 12–24 h post-dose was 0.15 (0.43) μg h^{-1}.

The mean (S.D.) cumulative amount of SCG excreted at 0.5, 1.0 and 24 h post dose for inhaled and oral dosing was 31.78 (15.42), 58.71 (31.09), 309.3 (162.73) mg and 3.93 (4.14), 5.99 (8.05), 66.31 (55.14) mg, respectively. The mean difference (95% confidence intervals) of the cumulative amount of SCG excreted at 0.5, 1.0 and 24 h post-dose (inhaled vs. oral) was 27.85 (13.32, 42.37), 48.81 (20.5, 77.1) and 238.5 (124.0, 353.1) mg, respectively. Urinary excretion of SCG 0–24 h post inhaled and oral dosing represents 1.55 and 0.33% of the dose, respectively.

The amount excreted in the urine varied considerably between different volunteers but the vari-

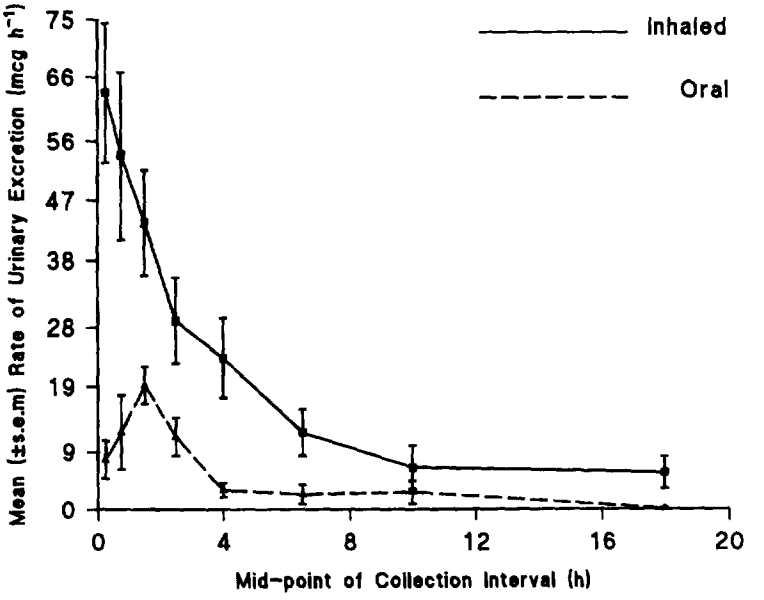

Fig. 2. Mean (±S.E.M.) rate of urinary excretion of SCG during the 24-h collection period post inhaled and oral dosing ($n=8$).

ability was consistent between collection periods. The inter-volunteer variability observed after oral therapy was approximately twice that following inhalation.

4. Conclusion

In this paper we have clearly demonstrated that both the quantitative and qualitative determination of SCG in urine can be achieved. The addition of dodecyl triethyl ammonium phosphate to the reversed-phase HPLC enables the adequate resolution of SCG and NCS from urinary interfering peaks. This method permits the detection of SCG in human urine at concentrations as low as 0.05 mg l^{-1} and may be used to determine SCG in urine samples from volunteers or patients collected up to 24 h after administration of 20 mg of the compound by inhalation.

The proposed method is precise and accurate. In contrast to those previously published, it provides a suitable, convenient and rapid (up to 40 samples can be analysed in one day) technique for the determination of SCG and NCS in urine samples from volunteers or patients, with adequate sensitivity and specificity. This method has been used routinely in our laboratory for over 12 months and has proved to be both consistent and reliable.

Radioimmunoassay studies have shown that SCG deposited in the respiratory tract is well absorbed [14]. Urinary excretion rate data from this study reveals that SCG is rapidly absorbed from the lungs after inhalation in contrast to the slow and incomplete absorption from the gastrointestinal tract. The mean urinary recovery of SCG, 0.5 and 1 h postdose, was 0.16 and 0.3% after inhalation, and 0.02 and 0.03% after oral dosing, respectively.

The urinary excretion of SCG at 0.5 and 1.0 h following inhalation can therefore be used to estimate the amount of drug reaching the respiratory tract. These results agree with those previously published by Auty et al. 1987 [15].

Approximately 50% of the absorbed SCG is excreted in the urine [3–5] and therefore with normal respiratory function and good inhalation technique, an average of 3.1% of the inhaled drug is deposited in the respiratory tract.

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