Journal of Chromatography, 305 (1984) 228—232
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1894

Note

Determination of sodium cromoglycate in human urine by high-performance liquid chromatography on an anion-exchange column

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(First received March 4th, 1983; revised manuscript received August 18th, 1983)

Sodium cromoglycate {disodium 5,5'-[(2-hydroxytrimethylene)dioxy]bis(4-oxo)-4H-1-benzopyran-2-carboxylic acid} which is also known as disodium cromoglycate and cromolyn sodium, is effective in the treatment of allergic diseases such as asthma [1]. The drug is a highly polar compound with two carboxylic acid groups (pK_a = 2). It is poorly absorbed orally [2] but is completely absorbed from the lung [3]. Sodium cromoglycate is not metabolised; it is cleared rapidly from the plasma and is excreted by man in both the bile and urine in approximately equal proportions [4]. Absorption of the drug by patients in the past has been assessed primarily by determination of the urinary excretion of the drug because of the difficulty of determining the low plasma concentrations following administration of therapeutic doses. Recently a sensitive radioimmunoassay method for the compound in plasma has been developed [5] which has sufficient sensitivity to allow the determination of these low plasma concentrations in patients.

Methods for the determination of sodium cromoglycate in urine include a colorimetric method [2], which was subsequently modified by the introduction of tritiated sodium cromoglycate as internal standard [6], a differential-pulse polarographic method [7] and a reversed-phase ion-pair high-performance liquid chromatographic (HPLC) method [8]. The former methods [2, 6, 7] involve sample concentration procedures and have limits of detection of 0.5 μg cm^{-3}. The latter method [8], which employs direct on-column injection of urine, has a limit of detection of about 0.35 μg cm^{-3}. This note describes an HPLC method which has a limit of detection of 0.05 μg cm^{-3} and employs an anion-exchange chromatographic column.

0378-4347/84/$03.00

EXPERIMENTAL

Reagents

Diethyl ether was purchased from May & Baker (Dagenham, U.K.). Concentrated hydrochloric acid, sodium chloride, glycine, and orthophosphoric acid (density 1.75 g cm^{-3}) were purchased from Fisons Scientific Apparatus (Loughborough, U.K.), and were analytical quality reagents. The mobile phase was prepared by dilution of 10 cm^3 of orthophosphoric acid to 1 dm^3 with pH adjustment using 5 *M* sodium hydroxide. This solution was 0.9 *M* with respect to phosphate.

Instrumentation

A Spectra-Physics (St. Albans, U.K.) Model 3500B high-performance liquid chromatograph was used. The stainless-steel column (250 × 4.6 mm) was obtained prepacked with Partisil SAX (particle size 10 μm) from Whatman Lab. Sales (Maidstone, U.K.). A Valco injection valve (Spectra-Physics) with a sample loop of 120 mm^3 was used. The mobile phase was phosphate buffer (pH 2.30 ± 0.01) delivered at a constant flow-rate of 3.6 cm^3 min^{-1}. Detection was made at 325 nm with a Schoeffel Model 770 variable-wavelength UV detector (Spectra-Physics), the output from which was recorded at 2 and 10 mV using a Vitatron 2001 twin-pen recorder (Fisons Scientific Equipment, Crawley, U.K.). The column was used at ambient temperature (approximately 22°C) and the column inlet pressure was about 12.5 MPa. Peak heights of sodium cromoglycate were routinely measured manually, but peak areas were also measured using a Columbia Scientific Instruments computing integrator (Kemtronix, Compton, U.K.) during the initial studies. The best straight lines were found by least-squares linear regression.

Sample preparation

Test urine (10 cm^3) was pipetted into glass 30-cm^3 disposable extraction tubes (Payne, London, U.K.) containing sodium chloride (5 g). Water (1.0 cm^3), concentrated hydrochloric acid (1.0 cm^3) and diethyl ether (10 cm^3) were added. Standards were prepared similarly using blank pooled 24-h urine except that aqueous sodium cromoglycate solutions (1.0 cm^3) were added in place of the water added to the test urine. Routinely, standards in the range 0.05—20 μg cm^{-3} were used. Each tube was capped with a plastic aluminium-lined screw-cap, was shaken for 10 min at 200 oscillations per min along the long axis, and was centrifuged at 1540 *g* for 10 min. The diethyl ether layer (9.0 cm^3) was removed and the extraction was repeated with diethyl ether (10 cm^3). The extracts were combined in a tube containing 1.0 cm^3 of 1 *M* glycine—hydrochloric acid buffer (pH 3.5). The tube was capped, shaken and centrifuged. Samples of the lower aqueous phase were injected onto the chromatographic column. Prepared samples could be stored as long as three days at ambient temperature without removal of the diethyl ether before being subjected to the chromatographic separation.

RESULTS

Chromatography

Chromatograms resulting from the analysis of a blank urine and blank urine containing sodium cromoglycate at concentrations of 0.2 and 1.0 $\mu g\ cm^{-3}$ are shown in Fig. 1. Chromatograms resulting from the analysis of urine from patients receiving sodium cromoglycate are shown in Fig. 2. The drug was eluted from the column at about 4.5 min as an asymmetric peak. The retention time was inversely dependent on the concentration of the phosphate in the mobile phase. The pH of the mobile phase also affected the retention of the drug; increasing pH from 1.9 to 5.4 resulted in shorter retention times.

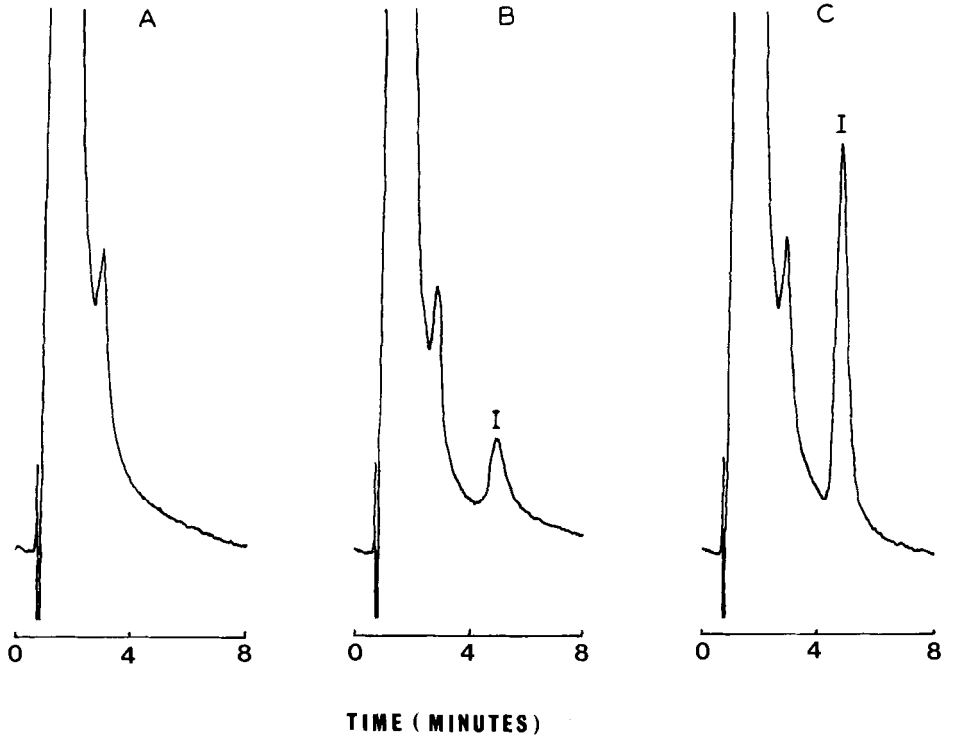

Fig. 1. Chromatograms of blank urine (A) and blank urine containing added sodium cromoglycate (I) at a concentration of (B) 0.2 and (C) 1.0 $\mu g\ cm^{-3}$.

Linearity

Peak area calibration curves were linear, for example, $y = 0.0794x - 0.003$, $r = 0.9977$, $n = 17$, over the concentration range 0.05—20 $\mu g\ cm^{-3}$. Peak height calibration curves were also linear over this concentration range ($y = 14.22x - 4.26$, $r = 0.9986$, $n = 17$) but the peak height data could be more exactly represented by two straight lines over the concentration ranges 0.05—1 and 1—20 $\mu g\ cm^{-3}$ which typically had the properties $y = 10.94x - 0.26$, $r = 0.9970$, $n = 7$ and $y = 14.71x - 10.44$, $r = 0.9988$, $n = 10$, respectively. Hence, whenever peak height measurments were used routinely two standard curves were constructed extending over the appropriate concentration range.

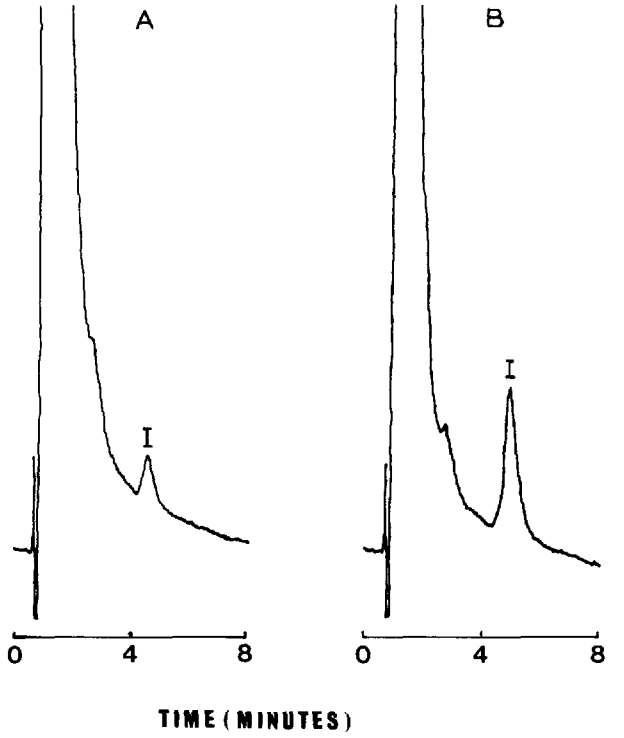

Fig. 2. Chromatograms of urine from patients collected over a 24-h period after administration of 20 mg of sodium cromoglycate (I) by inhalation. Concentration of sodium cromoglycate in samples: (A) 0.10 and (B) 0.34 $\mu g\ cm^{-3}$.

Precision, accuracy and recovery

The intra-day precision and accuracy of the method at sodium cromoglycate concentrationsof 0.22, 1.1 and 5.5 $\mu g\ cm^{-3}$ on two separate days are shown in Table I. The maximum coefficient of variation (C.V.) was 5.8% with a maximum inaccuracy of 11.8%. The efficiency of the extraction procedure is shown in Table II. The extraction efficiency was about 70% and was independent of concentration over the range 0.2—20 $\mu g\ cm^{-3}$.

TABLE I

INTRA-DAY PRECISION AND ACCURACY OF THE SODIUM CROMOGLYCATE METHOD ON TWO SEPARATE DAYS

Results obtained by analysis of six or more spiked urine replicates at each concentration on each day. Peak height determined.

Sodium cromoglycate concentration ($\mu g\ cm^{-3}$)	Coefficient of variation (%)		Accuracy (%)	
	Day 1	Day 2	Day 1	Day 2
0.22	2.3	1.7	99.6	98.2
1.1	4.6	5.8	103.9	111.8
5.5	2.8	2.4	96.2	100.5

TABLE II

EXTRACTION EFFICIENCY OF SODIUM CROMOGLYCATE FROM URINE ON TWO SEPARATE DAYS

Sodium cromoglycate concentration ($\mu g\ cm^{-3}$)	Extraction efficiency (%) (mean ± S.D.)	
	Day 1	Day 2
0.2	71.9 ± 4.2	66.7 ± 5.4
2.0	68.8 ± 7.4	79.7 ± 8.7
6.0	74.6 ± 2.8	Not done
20.0	70.5 ± 4.2	71.4 ± 2.0

Interference

No interfering peaks were observed at the retention time of sodium cromoglycate when urine from normal healthy volunteers or patients was examined. Similarly no interference occurred when drugs likely to be administered to patients concomitantly with sodium cromoglycate were chromatographed. The drugs studied included acetylsalicylic acid, sodium salicylate, phenylbutazone, prednisolone phosphate, hydrocortisone, paracetamol, terbutaline sulphate and theophylline. This latter investigation of the specificity of the method ignored any possible interference from metabolites of these drugs: the drugs were assessed by injection directly onto the chromatographic column.

CONCLUSION

The method described permits the detection of sodium cromoglycate in human urine at concentrations as low as 0.05 $\mu g\ cm^{-3}$ and may be used to determine sodium cromoglycate in urine samples from volunteers collected up to 24 h after administration by inhalation of 20 mg of the compound. A technician may analyse forty samples by the method in two days: automation of sample injection can improve this analysis rate substantially since this time estimate includes manual sample injection. The method has satisfactory accuracy and precision, is simple and has proved to be reliable in routine use during a period of more than three years.

ACKNOWLEDGEMENT

The author is grateful to Mrs. Jackie Jones for skilled technical assistance.

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