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Determination of Trifluoroacetic Acid and Chloride in Celecoxib Drug

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A method for the determination of trifluoroacetic acid and chloride in celecoxib drug by valve switching-ion chromatography was developed. Chromatographic separation was performed on an IonPac AS23 column. Mixture of 3.5 mmol/L sodium carbonate and 1.4 mmol/L sodium bicarbonate was used as mobile phase. Since celecoxib is poorly water-soluble (0.007 mg/mL), but extremely soluble in methanol (113.94mg/mL). Ion chromatographic columns are prone to fouling by strongly-retained organic sample components. In our research, column contamination caused by the drug molecule and organic solvent was avoided by using a valve-switching sample preparation method. The linear ranges of the method for the two analytes were 0.10-10 µg/mL and the detection limits (S/N = 3) of trifluoroacetic acid and chloride were 0.06 and 0.006 mg/L, respectively. This method was successfully applied to the determination of trifluoroacetic acid and chloride in real samples. The spiking recoveries of trifluoroacetic acid and chloride were in the range of 90 to 94 %.

Keywords: Ion chromatography, Valve-switching, Celecoxib drug, Trifluoroacetic acid.

INTRODUCTION

Celecoxib is a sulfa non-steroidal anti-inflammatory drug (NSAID) and selective COX-2 inhibitor used in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms and can also reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis[1,2]. It is synthesized by a Claisen condensation reaction of *p*-methylacetophenone with ethyl trifluoroacetate catalyzed by the sodium ethoxide to produce 1,3-dicarbonyl adduct. Condensation of the diketone with 4-sulfamoylphenyl hydrazine hydrochloride produces the 1,5-diarylpyrazole drug moiety (Fig. 1). In the synthetic process of celecoxib drug, ethyl trifluoroacetate and 4-sulfamoylphenyl hydrazine hydrochloride were used as reactants. As a result, the trifluoroacetic acid may be generated from hydrolysis of ethyl trifluoroacetate and chloride may be generated from 4-sulfamoylphenyl hydrazine hydrochloride. In the end, they are found in synthetic durg samples as impurities. Trifluoroacetic acid is a chemical that is corrosive and toxic and thus, it is imperative that any residual trifluoroacetic acid should be removed from the celecoxib prior of the final formulation. With relative to the chloride in celecoxib, the presence of chloride may not cause any adverse toxic effect. However, its presence may lower biological potency of celecoxib. Therefore, it is necessary that their impurities (trifluoroacetic acid and chloride) in bulk pharmaceutical chemicals or the final products were monitored to ensure its safety before it could be used.

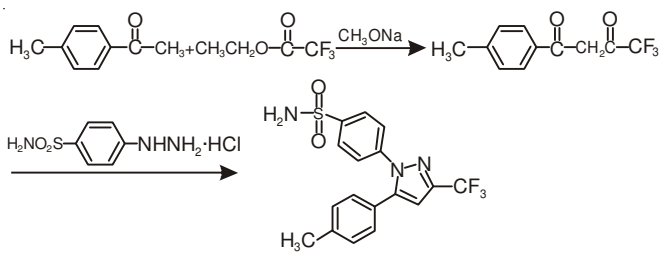

Fig.1. Synthetic route of the celecoxib

Up to now, various methods have been reported for the determination of trifluoroacetic acid in pharmaceutical samples, such as headspace-GC[3], GC-MSD[4], capillary electrophoresis[5-7] and, more commonly, ion chromatography[8-10]. GC and GC-MSD methods involved dissolving drug substance with methanol, in the presence of sulphuric acid, to convert the trifluoroacetic acid into the more volatile methyl ester. To our best of knowledge, ion chromatography (IC) is the most ideal analytical method for the analysis of trifluoroacetic acid and inorganic ions. However, celecoxib is poorly water-soluble

(0.007 mg/mL), but extremely soluble in methanol (113.94 mg/mL). Ion chromatographic columns are prone to fouling by strongly-retained organic sample components and it is important, in favor of method robustness, either to limit the amount of these materials injected into the column or to remove them from the column between sample injections using an organic eluent. The latter approach, however, has the disadvantage of extending the cycle time between injections owing to the need to re-equilibrate the column with aqueous mobile phase following the solvent rinse step. Lliterature[10] reported an avoiding column fouling method by removing the drug by alkaline precipitation before injection. The method performed robustly without the need of a column clean-up step between injections but need a manual operation to remove the drug molecule. Because of these current analytical limitations, we have to seek a new method for analysis of trifluoroacetic acid and chloride in celecoxib that samples can be injected directly to the separation column without column contamination and the need for manual sample preparation. There is a good method, which is valve-switching, has been successfully used for elimination of sample matrix and enrichment of analyte[11]. In this paper, samples were dissolved in organic solvent first, then water was added to dilute to suitable concentration. Using combination of short guard columns for analyte enrichment and column switching devices for organic removal offered a simple on-line sample preparation technique, which could be easily interfaced with the chromatographic system to develop high sensitivity assays. Using this on-line sample preparation approach, a sensitive and selective analytical method was developed for the simultaneous and direct quantitation of trifluoroacetic acid and chloride.

EXPERIMENTAL

Sodium tri-fluoroacetate (analytical grade) was supplied by Sigma-Aldrich, Schweiz, Buchs (St. Gallen), Switzerland. This reagent is hygroscopic and was stored in a desiccator when not in use. Methanol from Tedia Co. (Tedia, USA) was HPLC grade. Deionized water of 18.2 MO cm^{-1} achieved by a Millipore water system (Millipore, Mosheim, France) was used throughout. The standard stock solution (1000 mg/L) of chloride ion was purchased from Shanghai Institute of Metrology and Measurement. Other reagents used including anhydrous sodium carbonate and sodium hydrogen carbonate were of analytical grade. Working solutions of the target analytes were prepared by diluting stock solutions. Standards and spiking solutions were prepared by making suitable dilutions of an aqueous standard of sodium trifluoroacetate with deionized water. The concentration of these solutions was expressed in terms of the free acid.

The ion chromatographic analysis was performed on an ICS-1600 (Thermo Scientific, Waltham, MA, USA) equipped with a dual-piston pump, two six-port valve and a 25 mL sample loop. An eluent generator (ED40, Thermo Scientific, Waltham, MA, USA) with potassium hydroxide (KOH) cartridge and a CD20 conductivity detector (Thermo Scientific, Waltham, MA, USA) was used. The analytical column was an IonPac AS23(250 × 4 mm,I.D., Thermo Scientific, Waltham, MA, USA) and guard column was an IonPac AG23(50 × 4 mm, I.D., Thermo Scientific, Waltham, MA, USA). Another AG23(50 × 4 mm, I.D., Thermo Scientific, Waltham, MA, USA) guard column was used as the concentrator for analytes. The eluent was a mixture of 3.5 mmol/L sodium carbonate and 1.4 mmol/L sodium bicarbonate. The eluent flow-rate was set at 1 mL/min. All the instrument control and data acquisition were performed by Chromeleon 6.8 chromatography software (Thermo Scientific, Waltham, MA, USA).

Method development

Analytical procedure: The aim of sample preparation was to quantitatively recover analytes from the drug substance and to introduce the sample to the chromatographic system. However, robustness was not compromised according to our first work (Figs. 3 and 4). While the drug was dissolved in organic solvents and then directly injected into chromatograph. The baseline was not flat and there is a bump (solvent peak) in the middle. So, it was decided to try to remove organics before injection to the analytical column. As it is already noted that the celecoxib is poorly soluble in aqueous solution but readily soluble in methanol. A sample preparation method was therefore evaluated based on complete dissolution of the sample in a mixture of methanol and aqueous solution followed valve-switching to remove organic solvent and sample matrix. The operation of this valve-switching ion chromatography consists of three main steps (Fig. 2): sample loading, enrichment of the analytes and elimination of the organics and chromatographic separation. The first step: (valve load position for 0.3 min): a 25 µL injector loop was filled with drug sample achieved by over-filling at least greater than or equal to four times of the injector loop volume. The samples were filtered with 0.2 µm membrane filter before introduced into chromatograph. The second (valve injection position for 3 min): samples were eluted from sample loop by mobile phase (the mixed solution of methanol and water with a volume ratio of 1:4) with DXP pump (Thermo Scientific, Waltham, MA, USA) at a flow rate of 0.55 mL/min. The inorganics was concentrated in enrichment column while the organics were flushed out of enrichment column. The third, the valve position was returned to load. Chloride ion and trifluoroacetic acid concentrated in the enrichment column were separated on an analytical column using mobile phase mixture of 3.5 mmol/L sodium carbonate and 1.4 mmol/L sodium bicarbonate at a flow rate of 1.0 mL/min. A typical chromatogram of the drug sample was shown in Fig. 5. There was no bump on the baseline.

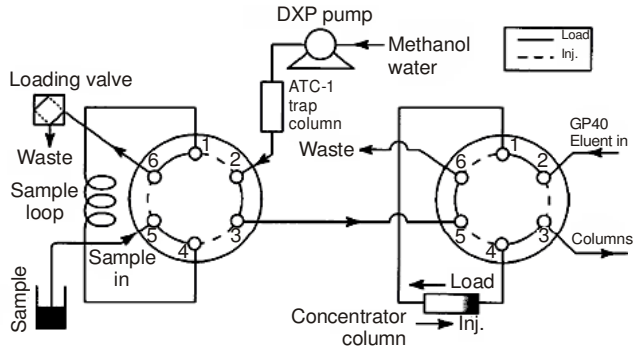

Fig. 2. A schematic diagram of the vavle-switching IC system. The arrows indicate the direction of the flow depending on the valve position

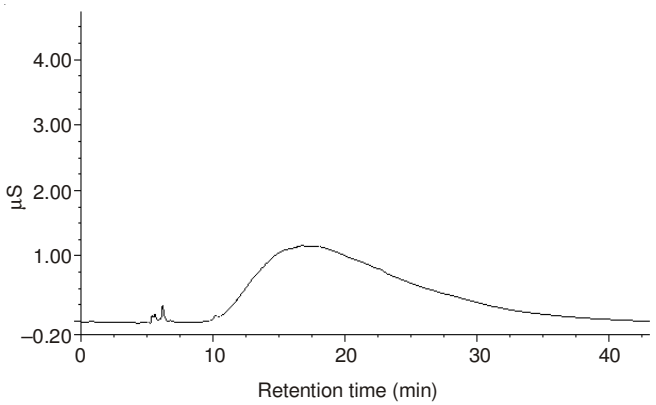

Fig. 3. Chromatogram of blank sample with direct injection into chromatography (without valve-switching)

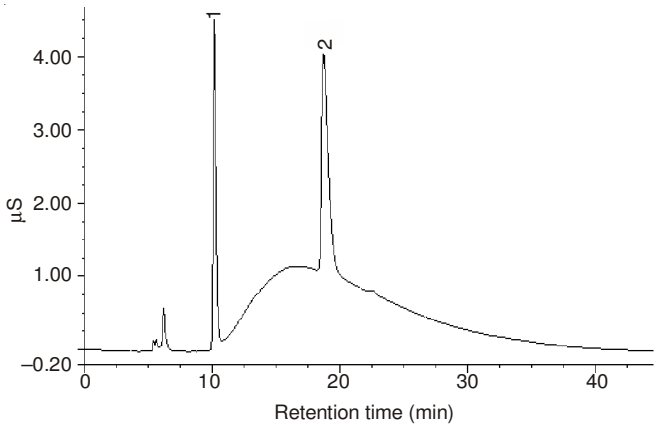

Fig. 4. Chromatogram of trifluoroacetic acid (9.6 µg/mL) and chloride (1.4 µg/mL) with direct injection into chromatography (without valve-switching)

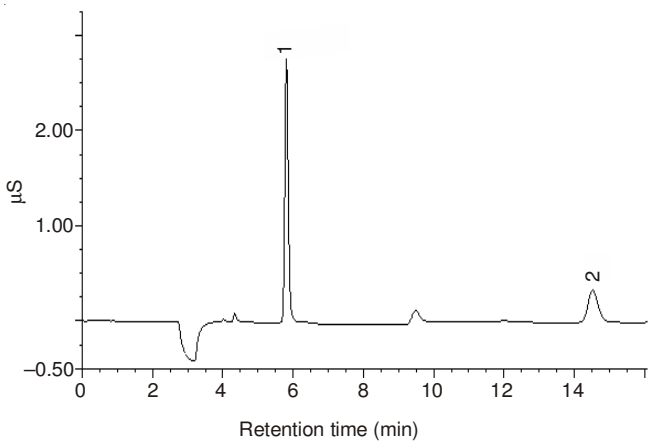

Fig. 5. Chromatogram of sample with direct injection into chromatograph (with valve-switching), 1-chloride (1 mg/L), 2-trifluoroacetic acid (1 mg/L)

RESULTS AND DISCUSSION

To obtain the optimized methanol concentration for complete drug dissolution, celecoxib solubility in the different volume ratios of methanol and water (1:1, 2:1, 4:1, 6:1, 8:1,10:1, v/v) were tested. The results showed that the drug was completely insoluble when the volume ratio was less than 1:4 (v/v). However, high volume ratio of methanol is not beneficial to our research, thus, the mixture of methanol and water at 1:4 (v:v) as a solvent of drug was a perfect choice.

Column selection: The efficiency of two types bonded stationary phase based quaternary ammonium alkyl or quaternary ammonium alcohol as functional group in the simultaneous determination of trifluoroacetic acid and chloride was evaluated. Due to strong polarization effect of trifluoroacetic acid, the strong hydrophilic quaternary ammonium alcohol (IonPac AS23) as functional group was more recommended and the result also indicated symmetrical peak shape, higher sensitivity. The elution of carbonate or potassium hydroxide systems can be used for IonPac AS23 column. Since elution of carbonate was more stable, after many tests, mixture of 3.5 mmol/L sodium carbonate and 1.4 mmol/L sodium bicarbonate as elution was selected and good separation was achieved (Fig. 6).

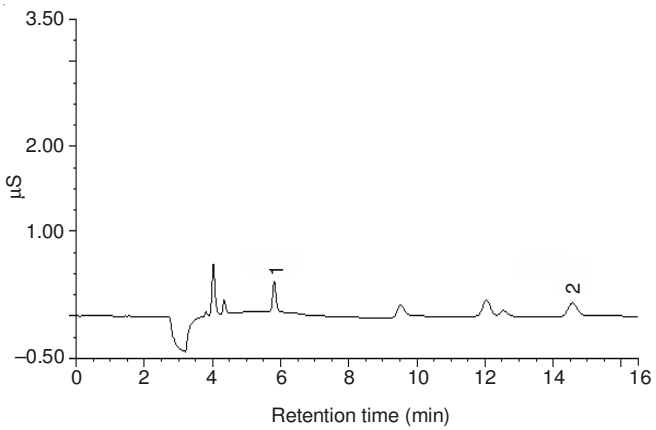

Fig. 6. Chromatogram of trifluoroacetic acid and chloride in pharmaceutical, 1-chloride, 2-trifluoroacetic acid

Method validation: The selectivity of the proposed method is satisfactory, because the peaks showed resolutions = 1.5 for the two determined compounds. The relative standard deviations (RSDs) of the retention times and peak areas for ten analysis within one day were less than 1.13 and 0.84 %, respectively. And inter-day RSDs of retention times and peak areas (5 days) were less than 0.28 and 2.75 %, respectively. The calibration curves were evaluated by plotting peak areas against the concentrations of anions. The linear calibration curves were obtained in corresponding concentration range. The correlation coefficients for analytes ranged from 0.9991 to 0.9993, showing good linearities obtained in two cases. The limit of detection (LOD) of trifluoroacetic acid and chloride was 0.06 and 0.006 µg/mL, respectively. It was defined as the detectable concentration of an anion giving a peak three-times as high as the background noise (S/N = 3), these parameters are shown Table-1. These values were enough for analysis of celecoxib.

TABLE-1
REGRESSION EQUATION, LINEAR RANGE AND CORRELATION COEFFICIENTS (r) OF CHLORIDE AND TRIFLUOROACETIC ACID

Analyte	Regression equation	r	Linear range (mg/L)	LOD (µg/L)	RSD_1 (%, n = 10)	RSD_2 (%, n = 5)
Chloride	Y=0.3137× 0.0028	0.9991	0.10-10	0.006	1.13	0.28
TFA	Y=0.1157×0.0005	0.9993	0.10-10	0.06	0.84	2.75

Y: peak area; x: mass concentration of analyte, mg /L; RSD_1: intra-day; RSD_2: inter-day; TFA: trifluoroacetic acid

Impurities in real samples: Trifluoroacetic acid and chloride were determined in a real sample using the proposed method. The chromatogram corresponding to one of the samples is shown in Fig. 6. The accuracy of the method was evaluated from recovery assays from preparing spiked samples of celecoxib. The obtained values ranged between 90 and 94 %. The RSD (n = 5) is below 2.53 %. All the contents data of the real sample were summarized in Table-2. The valve switching sample preparation method described here is simple and generally applicable to drugs with a similar solubility profile. The method performed robustly without the need for a column clean-up step between injections or manual-operating sample pretreatment. It is clear from Fig. 6 that the quality of the separation, in terms of peak shape and resolution, is still perfectly acceptable.

TABLE-2
CONTENTS AND RECOVERIES OF ANIONS IN THE SAMPLE

Analyte	Content	Sample added	Content	Recovery (%, n = 3)	RSD (%, n = 3)
Chloride	0.17	1.00	1.08	91	0.64
		0.20	0.35	90	
		0.10	0.26	90	
TFA	0.57	1.00	1.47	90	2.53
		0.50	1.04	94	
		0.20	0.75	90	

TFA = Trifluoroacetic acid

Conclusion

The method represents the advantage in the determination of the impurities in celecoxib. The method's performance is adequate to control trifluoroacetic acid and chloride as impurity in the drug substance. The valve-switching sample preparation technique employed succeeds in separating trifluoroacetic acid and chloride from the drug molecule; this is necessary because of the poor solubility of the drug in the mobile phase and the importance of avoiding contamination of the ion chromatography column with strongly-adsorbed organic species. This method has the potential to be applied generally to other drug molecules of a similar nature.

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