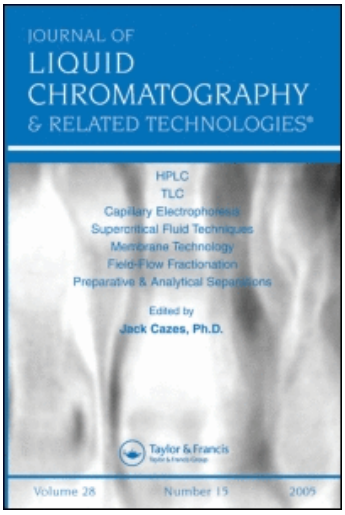

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LC-MS ANALYSIS OF SELECTED SULFUR-CONTAINING NON-STEROID ANTI-INFLAMMATORY AGENTS: APPLICATIONS TO PHARMACEUTICAL PRODUCTS

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ABSTRACT

A rapid, specific, sensitive, and reproducible high performance liquid chromatography-mass spectrometric assay has been developed for the quantitation of five sulfur-containing non-steroid anti-inflammatory drugs (NSAIDs), namely, celecoxib (Cel), piroxicam (Per), rofecoxib (Rof), sulindac (Sul), and tenoxicam (Ten), in available tablets and capsules. The examined compounds were extracted from the dosage forms with methanol and chromatographed on a Shim-Pack column using a mobile phase of acetonitrile and 1% acetic acid solution in a ratio 4:1 (Cel, Per, Sul, Ten) or acetonitrile and 20 mM ammonium acetate buffer solution (4:1) (Rof). The analytes were determined by an ion-trap mass spectrometer (Finnigan Mat) using APCI as an ionization process. The eluted compounds were detected either in the positive single ion monitoring mode at m/z 382.0, 332.0, 357.0, 338.4 (Cel, Per, Sul, Ten), or in the negative single ion monitoring mode at m/z 313.2 (Rof).

Linear correlations of the peak area and concentration were confirmed for all compounds over the concentration ranges 0.25-

1.0 μg /mL (Cel), 0.5-1.5 μg/mL (Per), 0.25-1.0 μg/mL (Sul), 1.0-2.0 μg/mL (Ten), and 0.1-1.0 μg/mL (Rof). The high specificity of the method was elucidated by analyzing mixtures of piroxicam/tenoxicam and celecoxib/sulindac.

The developed method was successfully applied to determine the examined drugs in marketed pharmaceutical products.

INTRODUCTION

Piroxicam is an anti-inflammatory agent which is prescribed in the treatment of chronic and acute inflammatory rheumatism and osteoarthiritis. Tenoxicam acts as an anti-inflammatory, analgesic, and also as inhibitor of platelet aggregation. Sulindac is a prodrug that is reversibly metabolized in humans into sulfide metabolite which possess a potent antirheumatic activity. The anti-inflammatory effect of the previous drugs is attributed to inhibition of prostaglandins biosynthesis by blocking both enzymes cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2). In this respect, adverse drug reactions such as gastrointestinal ulceration might occur during treatment with these medicines. Currently, celecoxib and rofecoxib are being safely recommended in rheumatic osteoarthiritis.

Pharmacologically, both drugs inhibit, specifically, the enzyme (Cox-2), which is present at high levels at the sites of inflammation. For quality assurance and quality control purposes (QA/QC), it is very important to establish accurate, sensitive, and selective analytical techniques that permit measurement of drug contents in dosage forms, and which can be further used in clinical investigations. Spectrophotometric,[1-3] spectrofluorimetric,[4] infrared spectrophotometric,[5] polarographic,[6] NMR,[7] and HPLC [8-10] techniques have been described for the analysis of piroxicam, sulindac, and tenoxicam in commercial products and biological samples.

The current procedures for the analysis of celecoxib and rofecoxib are very limited. A post-column derivatization HPLC procedure was only reported for the analysis of rofecoxib in human plasma.[11] LC-MS and LC-MS/ MS have been recently recognized as the techniques of choice for drug analysis due to robustness and high reliability.[12] This study reports on the application of LC-MS in the quantitative analysis of selected sulfur-containing NSAIDs in commercial pharmaceutical samples.

Data supporting the linearity, reproducibility, specificity, and limits of detection and quantitation are presented.

EXPERIMENTAL

Materials

Pharmaceutical grades of Cel, Per, Rof, Sul, and Ten, and their pharmaceutical products were used. HPLC grade solvents of methanol and acetonitrile were used for the extraction procedures and preparation of mobile phases. Water was purified by Milli-Q-System from Millipore Corporation. Other chemicals were of analytical grade.

Equipment

The LC-MS was composed of a high performance liquid chromatograph (Spectra P 2000) equipped with an ion-trap mass spectrometer (Finnigan Mat, USA). Chromatographic analyses were performed on a Shim-Pack GLC-CN column, 5 μm column (150 mm x 6 mm).

Two types of mobile phases at flow rates 1 mL/min were applied. The first one was composed of acetonitrile and 1% acetic acid solution (4:1) and was used for (Cel, Per, Sul, and Ten), whereas the second one was consisted of acetonitrile and 20 mM ammonium acetate solution (4:1) and was used for (Rof).

MS conditions were: APCI as an ionization process, vaporization temperature 450°C, scanning mode: positive (SIM), m/z 382.0, 332.0, 357.0, 338.4 (Cel, Per, Sul, and Ten) and negative (SIM), m/z 313.2 (Rof). Analytical data were acquired using LCQ software.

Calibration Curves

Standard stock solutions of Cel, Per, Sul, Ten, and Rof (10 μg/mL) were prepared by dissolving and diluting the appropriate amounts of the respective compounds in acetonitrile. Calibration curves were established by diluting 50-200 μL aliquots to 1 mL with mobile phase and injecting ~ 20 μL aliquot into MS detector using 20 μL loop size. The eluted compounds were monitored at the above m/z ratios.

All measurements were performed in duplicate for each concentration. The peak areas were automatically measured by LCQ software and were plotted versus drug concentrations. A least square linear regression analysis was used to determine the slope, Y-intercept, and the correlation coefficient of each calibration plot.

Sample Analysis

One tablet or capsule of the respective drug was transferred to a 100-mL volumetric flask and mixed with ~ 50 mL methanol. The mixture was sonicated for 30 min in an ultrasonic bath and diluted to 100 mL with methanol. After filtration of the solution through a membrane filter, an appropriate aliquot of the clear filtrate was diluted to 1 mL with mobile phase and 20 µL aliquot was injected. The percentage content of the NSAID in the commercial preparation was determined from the respective regression equation of the calibration curve.

RESULTS AND DISCUSSION

LC Conditions

A reversed phase HPLC in combination with a mass spectrometer has been developed for the analysis of selected sulfur-containing NSAIDs compounds. The mobile phase composition, as well as MS conditions, were properly selected to achieve optimum detection and quantitation. A mobile phase composed of acetonitrile and 1% acetic acid solution (4:1) (pH ~ 3.8) was found to be satisfactory for elution of Cel, Per, Sul, and Ten at relative short retention time 3.47-3.57 min. On the other hand, rofecoxib was eluted with a mobile phase composed of acetonitrile and 20 mM ammonium acetate buffer solution (4:1) (pH ~ 7) at retention time 3.44 min.

The high percentages of acetonitrile in both mobile phases permit rapid elution of compounds which is necessary required for QC purposes. The acidic pH of the first mobile phase allows detection of Cel, Per, Sul, and Ten in positive ion mode. The addition of ammonium acetate, at a concentration 20 mM to the second mobile phase, alter significantly, the pH which permits detection of Rof. in the negative ion mode.

The reproducibility of the retention time for each compound was checked by replicate injection of at least 10 samples of the analyzed compound. The relative standard deviation (RSD) was in the range 0.162 – 0.168 %.

MS Conditions

Mass spectrometric detection of the eluted sulfur-containing NSAIDs compounds was achieved by applying ion-trap technology using APCI as an ionization process. Preliminary investigations have shown that these compounds are good candidates for APCI. Based on the chemical structure (Figure 1) and physicochemical properties of the compounds, a positive ion scanning program was applied for detection of the positive molecular ions [MH^+] of Cel,

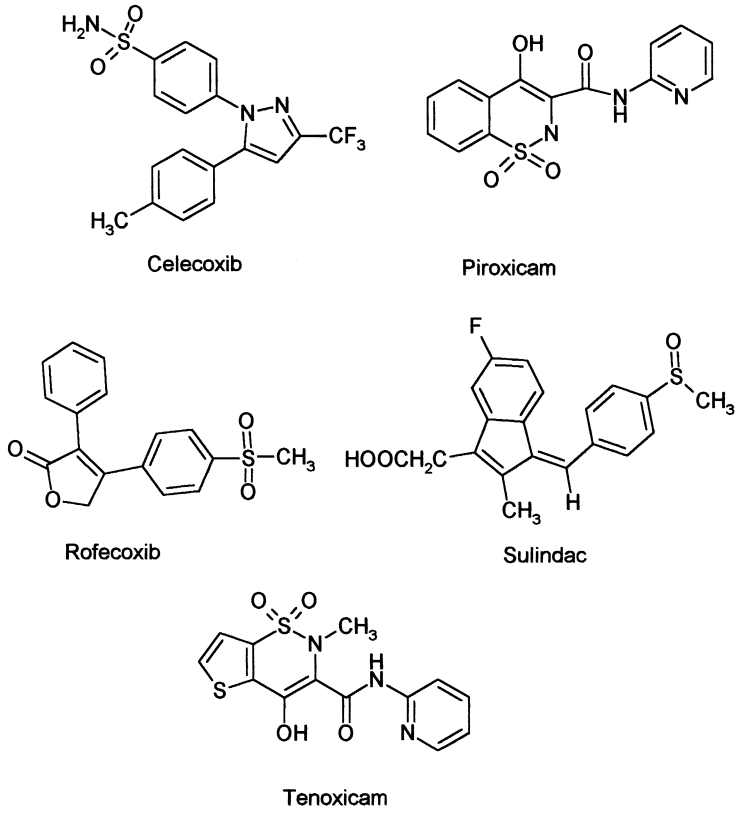

Figure 1. Structures of sulfur-containing NSAIDs.

Per, Sul, and Ten, whereas a negative ion scanning program was used for detection of the negative molecular ion $[M-H]^-$ of Rof.

Single ion chromatograms were extracted from the total ion chromatograms at m/z 382.0, 332.0, 357.0, 338.4, and 313.2 for Cel, Per, Sul, Ten, and Rof., respectively. These molecular ions were used for detection and quantitation purposes of the investigated compounds, either single or mixtures (Figures 2-4). A vaporization temperature of 450°C was selected for an optimum APCI ionization process.

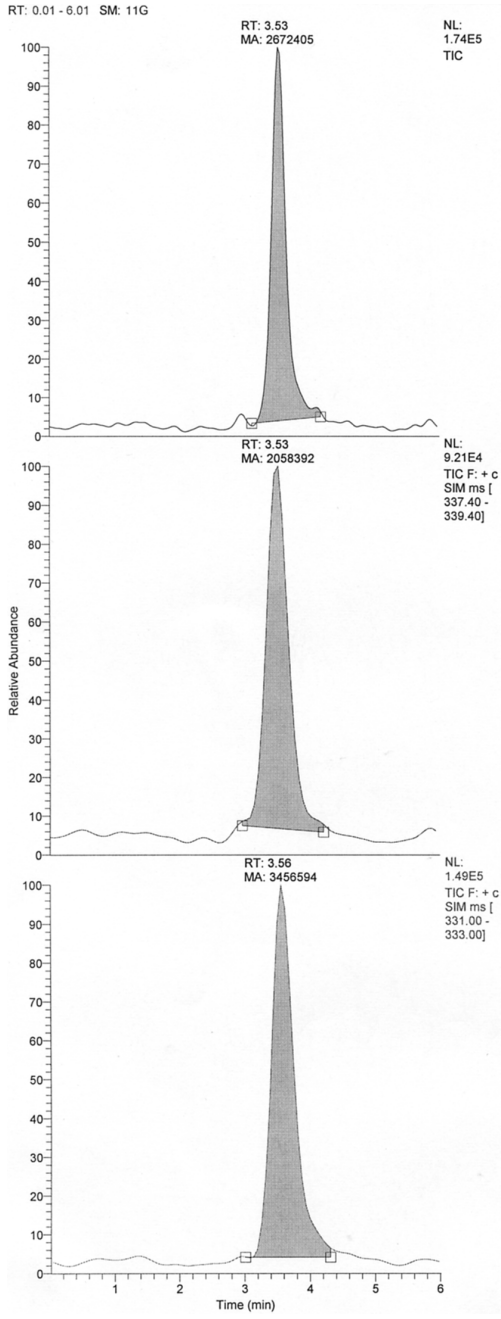

Figure 2. TIC and SIM chromatograms of mixture of piroxicam (1.5 μg/mL) and tenoxicam (2.0 μg/mL). Mobile phase: acetonitrile/1% acetic acid solution (4:1), molecular

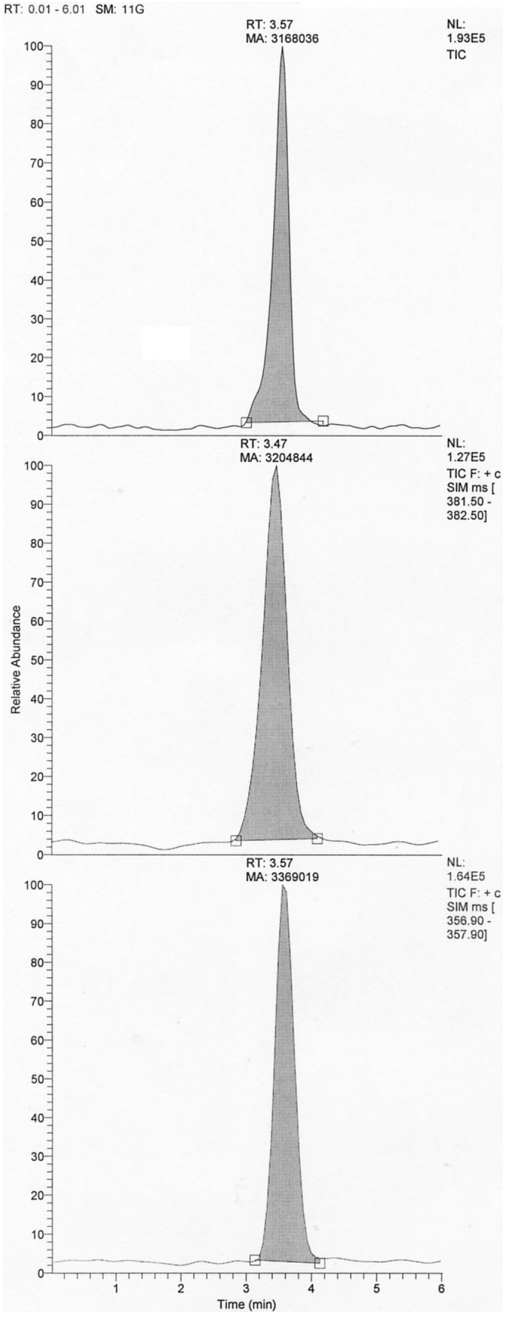

Figure 3. TIC and SIM chromatograms of mixture of celecoxib (1.0 μg/mL) and sulindac (0.5 μg/mL). Mobile phase: acetonitrile/1% acetic acid solution (4:1), molecular mass ions m/z 382.0 and 357.0.

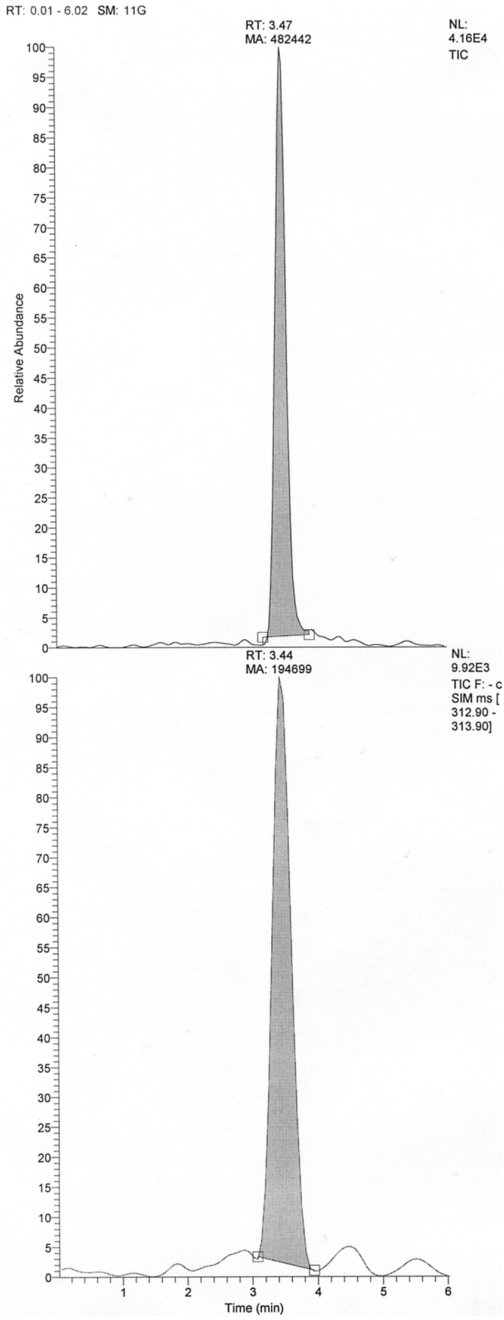

Figure 4. TIC and SIM chromatograms of rofecoxib (0.15 µg/mL). Mobile phase: acetonitrile/20 mM ammonium acetate solution (4:1), molecular mass ion m/z 313.3.

Linearity and Precision

The single ion chromatograms of NSAIDs exhibited well-defined peaks at the measured molecular mass ions. Quantitation was based on direct peak area measurement, which was automatically computed by the built-in LCQ software. Average calibration parameters for the standard plots with the respective concentration ranges were summarized in Table 1.

The reproducibility of LC-MS was assessed by constructing calibration plots for the individual components, under the same conditions, for 3 successive days (Table 2). Regression analyses of the data gave the respective slopes, intercepts, and regression coefficients. As shown, the data confirmed good linearity of the calibration curves and good reproducibility of the developed LC-MS method for the analysis of NSAIDs at these low concentration levels.

Limits of Quantitation and Detection

The limits of quantification for Cel, Per, Sul, Ten, and Rof were 0.05 μg/mL (Cel, Rof, Sul), 0.15 μg/mL (Per), and 0.5 μg/mL (Ten), respectively. The absolute detection limits for these compounds were 1 ng, 3 ng and 20 ng per 20 μL injection volume, respectively. The data suggest the application of the developed LC-MS for the quantitation of the sulfur-containing NSAIDs, particularly, celecoxib, rofecoxib, and sulindac in biological samples.

Table 1

Calibration Parameters for Standard Plots of Sulfur-Containing NSAIDs Using LC-MS Analysis

Compound	Concentration Range (μg/mL)	Regression Equation*	Correlation Coefficient
Celecoxib	0.25-1.0	$PA \times 10^{-5} = 0.54 + 27.60\ C$	0.9987
Piroxicam	0.50-1.5	$PA \times 10^{-5} = 1.06 + 23.99\ C$	0.9991
Sulindac	0.25-1.0	$PA \times 10^{-5} = -3.82 + 86.01\ C$	0.9989
Tenoxicam	1.0-2.0	$PA \times 10^{-5} = -0.99 + 11.81\ C$	0.9970
Rofecoxib	0.1-1.0	$PA \times 10^{-5} = 0.04 + 12.27\ C$	0.9995

* PA: Peak Area.

Table 2

Regression Analysis of Three Standard Plots* of Each of Sulfur-Containing NSAIDs Using LC-MS Analysis

Compound	Slope	Intercept	Correlation Coefficient
Celecoxib	28.88	+ 0.51	0.9999
	24.92	+ 0.68	0.9968
	29.01	+ 0.43	0.9994
Piroxicam	22.52	+ 1.40	0.9987
	24.43	+ 1.30	0.9989
	25.04	+ 0.47	0.9998
Sulindac	84.69	- 4.76	0.9984
	86.97	- 5.15	0.9989
	86.37	- 1.55	0.9999
Tenoxicam	10.75	- 0.52	0.9940
	11.18	- 0.83	0.9986
	13.50	- 1.61	0.9985
Rofecoxib	10.08	+ 0.03	0.9999
	12.59	+ 0.05	0.9998
	14.15	+ 0.03	0.9989

* $Pa \times 10^{-5} = a + b\,C$.

Table 3

Recovery Percentages of the Individual Components in Piroxicam/Tenoxicam and Celecoxib/Sulindac Mixtures Using LC-MS Analysis

Mixture (Comp I/ Comp II)	Comp I Nominal* Concen.	Comp I Calculated Concen.	Comp I %** ±SD	Comp II Nominal* Concen.	Comp II Calculated Concen.	Comp II %** ±SD
Piroxicam/ Tenoxicam	1.5	1.48-1.54	101.3 2.28	2.0	1.92-2.0	98.3 2.08
Celecoxib/ Sulindac	1.0	0.97-1.02	99.5 2.66	0.5	0.48-0.49	97.0 0.87

*Concentration (μg/mL). ** Mean of 5 determinations.

Specificity

As LC-MS relies on the detection of either positive or negative molecular ions of the analytes, irrespective of their UV absorption properties, therefore, it is possible to detect one component in the presence of the others, even when the elutes are not completely resolved. The respective chromatogram can be extracted from the total ion chromatogram at a specific molecular mass ion using a SIM scanning program. Thus, pairs of piroxicam/tenoxicam, and celecoxib/sulindac were identified (Figures 2,3) and quantified (Table 3). Percentages of the individual components were determined from regression equations of the calibration curves carried out simultaneously.

Method Application

The developed LC-MS method was applied to measure the drug contents of Cel, Per, Sul, Ten, and Rof in commercially available dosage forms such as tablets and capsules. Table 4 displays the percentages of the claimed contents and RSD of drug measurement for each product. The results showed satisfactory recovery percentages of the sulfur-containing NSAIDs compounds in dosage forms. The results are significantly valuable, particularly, for the QC/QA of celecoxib and rofecoxib in pharmaceutical samples, as no analytical methods for the quality control of these drugs in dosage forms were reported.

Table 4

Determination of Sulfur-Containing NSAIDs in Pharmaceutical Preparations

Compound	Pharm. Forms	Claimed Content	% Claimed Content (Mean ± SD)	RSD%
Celecoxib	Capsules[a]	100 mg	98.6 ± 2.4	2.43
Piroxicam	Capsules[b]	10 mg	102.2 ± 3.1	3.09
Rofecoxib	Tablets[c]	25 mg	97.6 ± 1.8	1.84
Tenoxicam	Tablets[d]	20 mg	98.3 ± 2.6	2.64

[a] Celebrex®, Pfizer/Egypt, under the authority of Searle Co., USA, MFD 12/99, Exp 9/2001. [b] Feldene®, Pfizer/France, under the authority of Pfizer, USA, MFD 11/99, Exp 11/2000. [c] Vioxx®, packed by Global Napi Pharmaceuticals/Egypt, under license from Merck Co., USA, MFD 12/99, Exp 12/2001. [d] Tilcotil®, Hoffmann La-Roche Ltd., Basel/Switzerland, MFD 7/98, Exp 7/2001.

CONCLUSIONS

The newly developed LC-MS procedure presented in this article was demonstrated to be fast, precise, and convenient for the determination of celecoxib, piroxicam, rofecoxib, sulindac, and tenoxicam, either individually or in mixtures. The method is highly selective, sensitive, and permit quantitation of the compounds in pharmaceutical preparations using single-step extraction procedure.

The reported method is useful for routine analysis and quality control of sulfur-containing NSAIDs. The developed LC-MS method contributes specifically to the analytical techniques for the determination of celecoxib and rofecoxib, which are significantly lacking.

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