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Determination of diclofenac sodium, flufenamic acid, indomethacin and ketoprofen by LC-APCI-MS

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Abstract

A sensitive, selective and accurate high-performance liquid chromatography-mass spectrometry (LC-MS) assay for the determination of selected non-steroidal anti-inflammatory drugs (NSAIDs), namely diclofenac sodium (DIC), flufenamic acid (FLU), indomethacin (IND) and ketoprofen (KET), either individually or in mixtures, was developed. The examined drugs were injected onto Shim-pack GLC-CN column and were eluted with a mobile phase consisting of acetonitrile and 20 mM ammonium acetate solution (5:1 v/v)/pH 7.4 at a flow rate 1 ml min^{-1}. The mass spectrometer, operated in the single ion monitoring mode, was programmed to admit the negative ions $[M-H]^-$ at m/z 295.9 (DIC), 280.1 (FLU), 355.8 (IND) and 252.9 (KET), respectively. The calibration curves were linear ($r \geq 0.9993$) over the concentration range 50–300 ng ml^{-1} (FLU, DIC) and 100–500 ng ml^{-1} (KET, IND) with detection limits of 0.5–4.0 ng. The mean predicted concentrations for the analytes were in the range −5.9 and 5.2% of the nominal concentrations. Within-day and between-day precision were in the range of 0.8–9.1% of the R.S.D. Mean recovery percentages of the individual compounds from laboratory-made mixtures and pharmaceutical formulations were (99.5–101.5%) and (100.6–102.2%), respectively.

Keywords: Non-steroidal anti-inflammatory drugs; LC-MS; Pharmaceutical analysis

1. Introduction

Diclofenac sodium, flufenamic acid, indomethacin and ketoprofen are well-known representatives of NSAIDs. These compounds are clinically prescribed as antipyretics, analgesics and anti-inflammatory agents. The antipyretic effect is due to a resetting of the hypothalamic temperature-regulating center, whereas the anti-inflammatory and analgesic effects are due to inhibition of prostaglandin synthesis. Therapeutically, NSAIDs are indicated to control pain and inflammation, however, the therapeutic efficacy, adverse reactions and tolerability are considerably variable. For clinical studies and drug quality control purposes, it is essential to establish accurate, sensitive and selective analytical techniques that permit

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detection and quantitative measurement of drug entities in biological and pharmaceutical samples. Spectrophotometric [1–5], spectrofluorimetric [6,7], polarographic [8], conductometric [9], NMR [10], high-performance liquid chromatographic [11–13], high-performance thin layer chromatography [14], gas chromatographic [15], GC/MS [16–18] and capillary electrophoresis [19,20] were reported. Although the chromatographic methods gave reproducible and reliable results, however, these methods require optimum resolution of the analytes from formulation excipients or biological matrices before being analyzed. Recently, LC-MS has been used in clinical and pharmaceutical analyses as it provides better efficiency of drug detection, characterization and quantitation [21,22]. Despite of LC-MS complexity, however, the instrument software permits smooth utility and applications. Generally, LC-MS permits low level detection and quantitation of drugs with high degree of specificity at relatively short-time of analysis without a need of complete chromatographic resolution of analytes. This paper reports on the application of LC-MS for the determination of selected NSAIDs such as DIC, FLU, IND and KET by monitoring the negative molecular ions of the examined compounds in SIM mode, using APCI as an ionization process. The higher specificity of the method is demonstrated by analyzing quaternary mixtures of NSAIDs. The utility of LC-MS for the analysis of representative pharmaceutical products of DIC, IND and KET is also reported.

2. Experimental

2.1. Chemicals and reagents

DIC, FLU, IND and KET were obtained from Sigma Co (St. Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from APS Ajax Finechen, Australia. Water was purified by milli-Q-System from Millipore Corp. (Milford, MA, USA). Other chemicals were of analytical grade.

2.2. HPLC and APCI-MS condition

HPLC analyses were performed on a Shimpack GLC-CN, 5 μm, column (150 × 4 mm), using Spectra P 2000 HPLC system. The mobile phase consisted of acetonitrile and 20 mM ammonium acetate solution (5:1 v/v)/pH 7.4. The flow rate was 1 ml min^{-1}. Injection of samples was manually done using 10 μl-loop size. Mass spectrometric detection was carried out using LCQ Mass Spectrometer, Finnigan MAT (USA) operated in the negative APCI mode. The mass spectrometer was programmed to admit the deprotenated molecules $[M-H]^-$ at mass to charge ratio (m/z) of 295.9 (DIC), 280.1 (FLU), 355.8 (IND) and 252.9 (KET), respectively. The vaporization temperature was set at 200 ± 1°C. The molecular ions were monitored in single ion monitoring (SIM) mode and the analytical data were acquired by LCQ software.

2.3. Standard solutions

Stock solutions of DIC, FLU, IND and KET at concentration 1 μg μl^{-1} were prepared in methanol. Working standards were prepared at concentrations 10 ng μl^{-1} in the mobile phase.

2.4. Calibration curves

Five standard solutions of each drug were prepared in concentration ranges 50–300 ng ml^{-1} (FLU, DIC) and 100–500 ng ml^{-1} (KET, IND) in the mobile phase. A 10-μl aliquot of each solution was injected onto the LC-MS system. A calibration curve for each compound was constructed by plotting the peak area of the analyte against concentration. Peak area measurements were simultaneously determined by LCQ software.

2.5. Assay validation

2.5.1. Calibration

Linear regression analysis was used to determine the regression equation representing the calibration curve for each compound.

2.5.2. Accuracy and precision

The accuracy and precision (within-day and between-day) for NSAIDs were determined by replicate analysis of control samples containing the drug at concentrations 50 and 300 ng ml^{-1} (DIC, FLU) and 100 and 300 ng ml^{-1} (IND, KET) for 5 successive days.

2.6. Sample analysis (recovery studies)

2.6.1. Laboratory-made mixtures

Quaternary laboratory-made mixtures of the NSAIDs were prepared by mixing ≈ 50 mg of each of DIC, FLU, IND and KET with ≈ 200 mg of excipients mixture (lactose, starch and magnesium trisilicate). The mixtures were transferred into 100-ml volumetric flasks, mixed with 50 ml methanol, sonicated for 30 min and then diluted to volume with methanol. A 20-ml portion of the clear filtrate was collected. Aliquots were suitably diluted with mobile phase to obtain concentrations of the individual components in the mixtures at 300 ng ml^{-1}. A 10-μl aliquot was injected and analyzed as above. The concentrations were determined from the calibration curves and the recovery percentages were calculated by reference to nominal concentration values.

2.6.2. Dosage forms

The crushed powder of one tablet or the content of one capsule was transferred into 100-ml volumetric flask, mixed with ≈ 50 ml methanol, sonicated for 30 min and then diluted to volume with methanol. The mixtures were filtered and the collected filtrates were clarified using membrane filters. Aliquots were suitably diluted with mobile phase and analyzed using the developed LC-MS. The drug contents were determined from regression equations of the calibration curves and the recovery percentages were calculated by reference to the labeled amounts.

3. Results and discussion

Recently, the utility of LC-MS in the analysis of drugs in biological and pharmaceutical samples has been increased dramatically. The inclusion of atmospheric pressure ionization (API) has improved the sensitivity, robustness and ease-of-use of LC-MS. Both API techniques, electrospray ionization (ESI) and atmospheric chemical ionization (APCI) are very 'soft' ionization modes that produce positive and negative molecular ions from polar molecules [23]. The molecular ions can be used to identify the analytes in the qualitative analysis and may be specifically monitored to provide quantitative data. Unlike the classical HPLC with UV detection, LC-MS permits determination of drugs in mixtures, even when they are incompletely resolved. Applications of LC-MS for the analysis of adenosine nucleosides in the presence of their hydrolytic products [24] and determination of mixtures of selected antiepileptics and beta-blocking drugs [25], were reported by the authors.

3.1. Method development

Before the application of LC-MS to the quantitative analysis of NSAIDs in pharmaceutical samples, it is quite important to adjust the chromatographic conditions. The composition as well as pH of the mobile phase were controlled. A mobile phase composed of acetonitrile and 20 mM ammonium acetate solution in a ratio 5:1 v/v at pH 7.4 was found to be suitable to ensure complete conversion of the weak acidic non-ionized molecules of NSAIDs into the negatively charged ions that can be detected. The high percentage of acetonitrile in the mobile phase permit detection of NSAIDs at low retention time (< 5 min) at a flow rate 1 ml min^{-1}. At acidic pH (≈ 3.5), the eluted compounds were poorly detected by MS detector. Addition of a volatile salt such as ammonium acetate to the mobile phase will alter the pH to slightly basic value (pH 7.4) that permits MS detection of the analytes with an adequate degree of sensitivity and specificity. Under the selected chromatographic conditions, the negative molecular ions $[M-H]^-$ of the resolved NSAIDs were detected at a vaporization temperature of 200 ± 1°C. At higher vaporization temperatures (430–450°C), the negative molecular ions were poorly detected due to fragmentation of the molecular ions. The deprotenated molecular ions

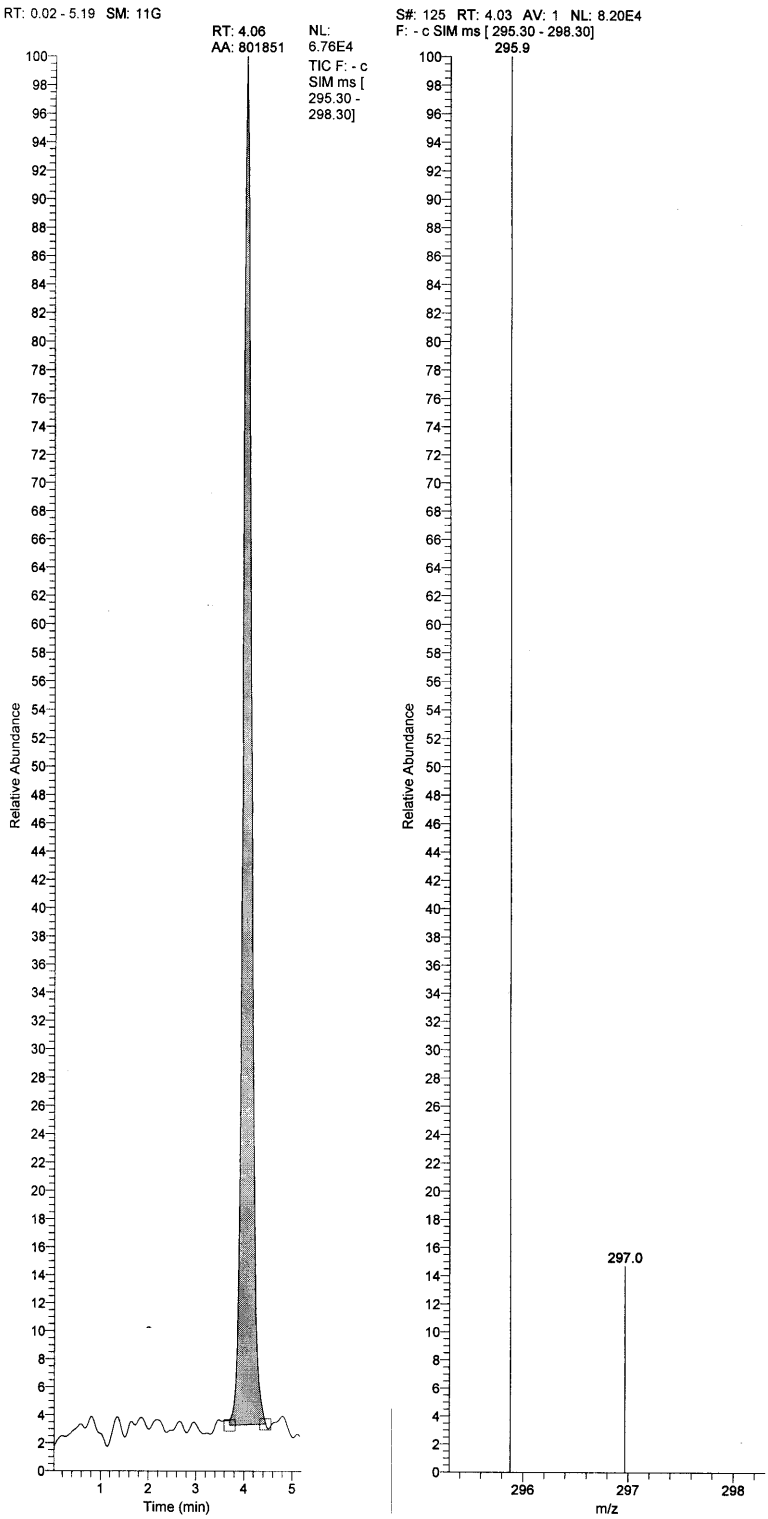

Fig. 1. Representative LC-MS of DIC (300 ng ml^{-1}).

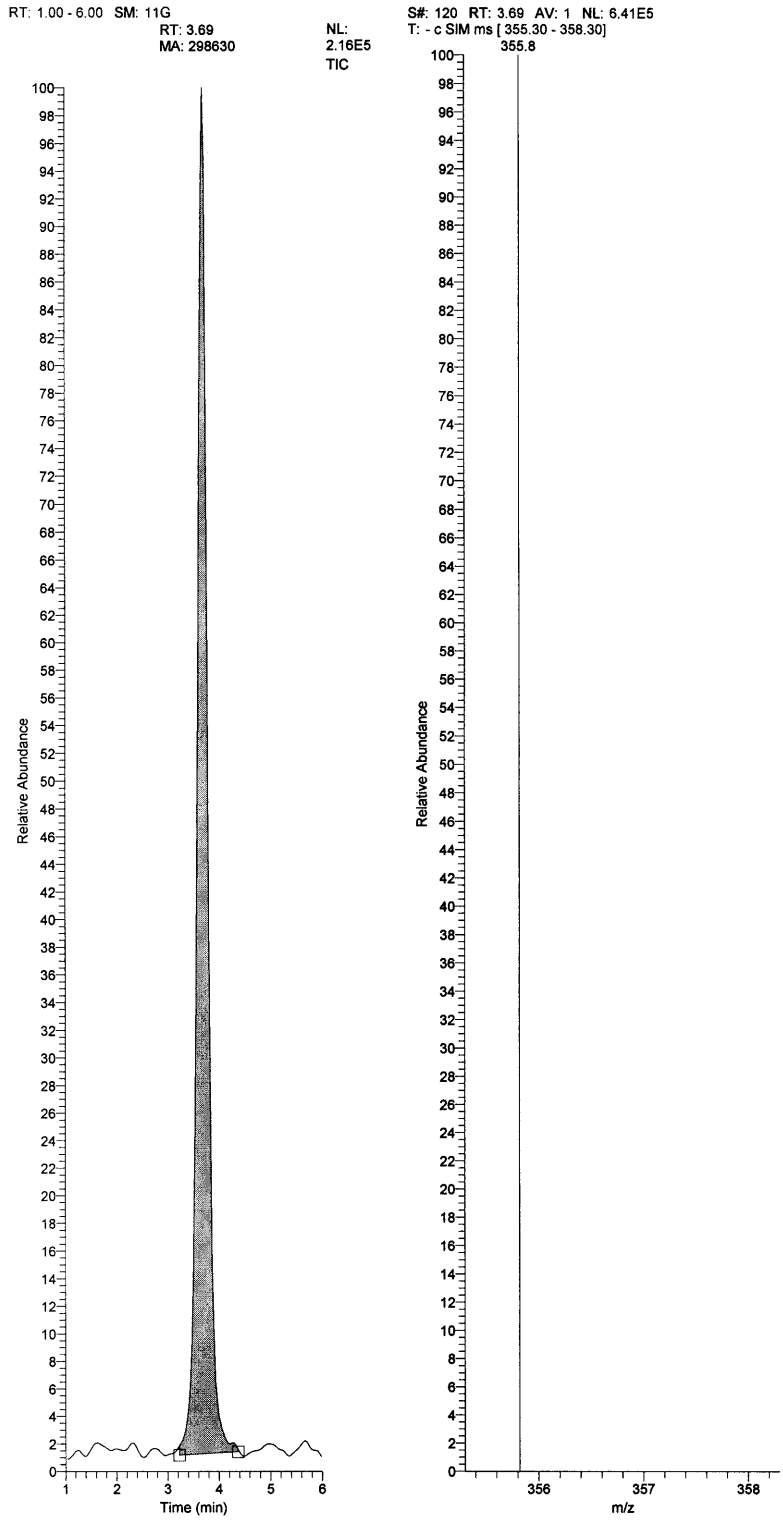

Fig. 2. Representative LC-MS of IND (500 ng ml^{-1}).

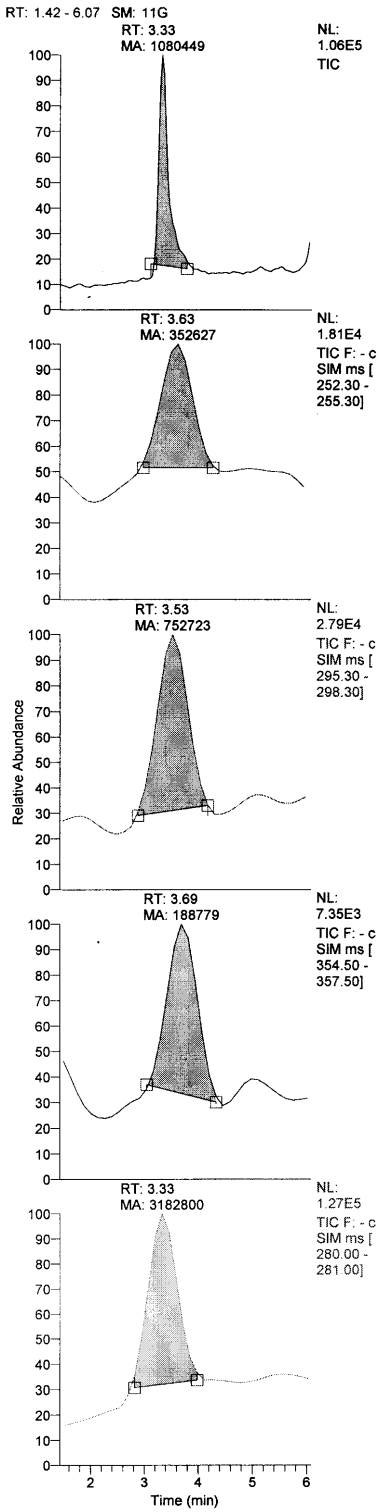

Fig. 3. Total ion chromatogram (TIC) of a quaternary mixture of KET, DIC, IND and FLU and the extracted SIM chromatograms of the individual components (300 ng ml^{-1} of each).

at m/z 295.9, 280.1, 355.6 and 252.9 were selected for the quantitation of DIC, FLU, IND and KET, respectively. Individual LC-MS chromatograms of DIC and IND were presented (Figs. 1 and 2). To prove the specificity of LC-MS, quaternary mixtures of NSAIDs were analyzed according to a designed scanning program. The SIM chromatograms of the individual components were extracted and recognized from the TIC chromatogram of the mixture (Fig. 3). As shown, the analyzed compounds were detected and quantified at a very narrow retention time range (< 0.5 min), which is not feasible using the classical HPLC.

3.2. Assay validation

3.2.1. Linearity

Quantitation of the individual NSAIDs was based on direct peak area measurements that were automatically computed by LCQ software. Using linear regression analysis, the peak area values were linear over the selected concentration ranges (Table 1). The regression coefficients were in the range 0.9976–0.9997 and the slopes were consistent with the measured peak area. The calibration concentration ranges were relatively lower compared with other analytical techniques such as HPLC due to high sensitivity of LC-MS. This might suggest the application of LC-MS to the analysis of NSAIDs in small-sized pharmaceutical samples and in biological fluids.

3.2.2. Limits of detection and quantitation

The lowest concentrations of NSAIDs to be detected were found to be 0.5 ng (FLU), 1.0 ng (DIC, KET) and 4.0 ng (IND), respectively. The lowest quantitation limits that gave $\approx 10\%$ R.S.D. were 50 ng ml^{-1} (FLU, DIC) and 100 ng ml^{-1} (KET, IND), respectively.

3.2.3. Accuracy and precision

The accuracy, expressed as % deviation of the nominal concentrations (%DEV), ranged from −

Table 1
Calibration curve parameters for the determination of NSAIDs by LC-MS

Analyte	Concentration range[a] (ng ml^{-1})	Regression equation[b]	Regression coefficient
FLU	50–300	$PA \times 10^{-3} = 3.5 + 10.8C$	0.9997
DIC	50–300	$PA \times 10^{-3} = 11.7 + 2.5C$	0.9976
KET	100–500	$PA \times 10^{-3} = -3.3 + 1.2C$	0.9996
IND	100–500	$PA \times 10^{-3} = -4.7 + 0.6C$	0.9993

[a] $PA = a + bC$ (PA, peak area; a, intercept; b, slope).
[b] Calibration points: five, each in duplicate.

Table 2
Accuracy, within-day and between-day precision of LC-MS for the determination of NSAIDs

Analyte	Nominal concentration (ng ml^{-1})	Calculated concentration[a] (ng ml^{-1})	%DEV[b]	%R.S.D.[c]	
				Within	Between
DIC	50	47.1	−5.9	5.8	7.6
	300	297.4	−0.9	1.7	2.6
IND	100	95.5	−4.5	2.0	8.9
	300	305.0	1.7	0.8	3.1
FLU	50	48.1	−3.8	2.0	9.1
	300	304.7	1.6	2.9	4.3
KET	100	105.2	5.2	3.4	7.6
	300	298.0	−2.0	1.8	3.6

[a] Mean of five determinations.
[b] %DEV, % deviation from nominal value.
[c] %RSD, % relative standard deviation.

5.9 to 5.2%. The within-day and between-day precision, expressed as % R.S.D., were 0.8–5.8 and 2.6–9.1%, respectively. The results were summarized in Table 2. The data indicated good accuracy and precision of LC-MS for the determination of NSAIDs at these low levels and suggested the application of the developed method for the analysis of the investigated compounds in pharmaceutical samples.

3.3. Recovery studies

Recovery percentages of the individual NSAIDs in quaternary laboratory-made mixtures were calculated by comparison with the nominal concentrations. Mean recovery percentages of 99.5–101.5% were computed (Table 3). The data proved that the LC-MS can be successfully used to analyze complex mixtures without complete chromatographic separation (HPLC,GC) or preliminary mathematical treatment (UV spectrophotometry, spectrofluorimetry). To indicate the potential of LC-MS for quality control and routine analysis of NSAIDs in dosage forms, the tablets of DIC and KET and the capsules of IND were analyzed. The LC-MS permit the analysis of NSAIDs in dosage forms by recognizing the

Table 3
Recovery percentages of NSAIDs from quaternary laboratory-made mixtures ($n = 4$) using LC-MS

Analyte	Nominal concentration (ng ml^{-1})	Recovery % ± S.D.[a]
DIC	300.0	100.7 ± 3.4
FLU	300.0	101.5 ± 3.2
IND	300.0	101.3 ± 4.8
KET	300.0	99.5 ± 1.4

[a] Mean of four determinations for each component in mixtures.

Table 4
Recovery percentages of DIC, IND and KET in commercial samples using LC-MS

Analyte	Labeled concentration	Recovery % ± S.D.[a]
DIC[b]	50 mg/tablet	100.9 ± 1.7
IND[c]	25 mg/capsule	100.6 ± 0.8
KET[d]	100 mg/tablet	102.2 ± 1.6

[a] Mean of five determinations.
[b] Clofen®/50 mg diclofenac sodium per tablet, Gulphar, U.A.E.
[c] Indocid®/25 mg indomethacin per capsule, MSD, Netherlands.
[d] Profénid®/100 mg ketoprofen per tablet, Specia Rhône-Poulenc Rorer, France.

molecular masses and determining the drug entities. Mean recovery percentages of 100.6–102.2% were estimated, which were in good agreement with the labeled amounts (Table 4).

4. Conclusions

A rapid and precise LC-MS procedure based on single ion monitoring of the negative molecular ions of DIC, FLU, IND and KET was developed and validated. The reported method provides several advantages such as high specificity, unnecessary complete separation of analytes, non-tedious sample preparation and obvious short run-cycle time. The higher sensitivity and selectivity of LC-MS suggests an extension of the method in routine analysis of NSAIDs in complex matrices such as biological fluids.

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