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Spectrofluorometric determination of montelukast in dosage forms and spiked human plasma

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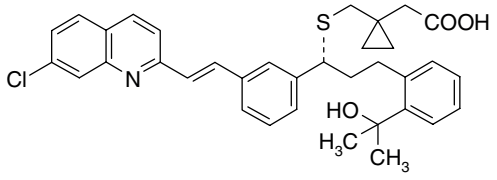
The native fluorescence of montelukast has been studied under different experimental conditions. The highest fluorescence intensity was obtained in methanol at 390 nm using 340 nm for excitation. Surfactants and sensitizers had either a negative or a slightly positive effect on its fluorescence intensity. The fluorescence intensity-concentration plot was rectilinear over the range 0.125 to 5 μg/ml with a lower detection limit of 0.02 μg/ml (3.4×10^{-8} M). Interference likely to be introduced from co-formulated drugs (such as loratadine) or co-administered drugs (such as verapamil, carbazepam, propranolol) or other common drugs, was studied. The method was successfully applied to the determination of the drug in tablets (pediatric tablets, chewable tablets and adult tablets). The mean % recoveries were in agreement with those provided by the manufacturer. The method was further applied to the *in vitro* determination of montelukast in spiked human plasma, the mean % recovery ($n = 5$) was 100.08 ± 1.40.

1. Introduction

Montelukast (MKST) is a potent and selective antagonist of the cysteinyl leukotriene receptor, used for the treatment of asthma. Leukotriene inhibitors (anti-LTS) are a new pharmacological class of compounds for asthma management. Their discovery had a significant impact on treatment strategies for the management of asthma (Cylly et al. 2003). It can be administered orally once daily, thereby increasing compliance over other common asthma treatments, it has almost no adverse effects or drug interactions, it has demonstrated efficacy against allergy or exercise induced bronchoconstriction, and is the only leukotriene modifier approved by the US Food and Drug Administration for use by children from 2–12 years of age (Knorr et al. 1998). A rapid onset of action is seen after the administration of MKST sodium, with improvements seen on the first day of treatment, and these positive effects may be additive to those of inhaled corticosteroids (Laviolette et al. 1999).

Reviewing the literature revealed that, all the methods reported for the assay of montelukast, whether in pharmaceutical preparations or in biological fluids rely on the use of chromatography, viz HPLC (Alsarra 2004; Amin et al. 1997; Ochiai et al. 1998; Liu et al. 1997; Al-Rourthi et al. 2004; Kitchen et al. 2003, Radhakrishma et al. 2003) and HPTLC (Sane et al. 2004). Although chromatographic methods have a high degree of specifity, yet, sample clean up and the instrumentation limitations preclude their use in routine clinical studies. The literature reveals no reports on the fluorometric determination of MKST. This led us to study the fluorescence characteristics of the drug as an attempt to develop a simple, sensitive and reliable method for its determination in dosage forms and plasma. The native fluorescence of the compound initiated the present study. The method was developed as an alternative to HPLC methods, and the results obtained were promising.



2. Investigations, results and discussion

The UV spectrum of MKST in methanol shows several λ_{max} values. The most prominent one is that at 344 nm with a specific absorbance [$A^{1\%}_{1cm}$] of 464 and molar absorptivity (ε_o) of $2.72 \times 10^4 \cdot mol^{-1} \cdot cm^{-1}$. As a consequence poor sensitivity will be achieved by conventional spectrophotometric measurements, and this problem is more aggravated if the compound needs to be estimated in biological fluids. On the other hand, MKST solution exhibited an intense native fluorescence. Different media, such as water, methanol, 0.1 M HCl and 0.1 M NaOH were attempted. As shown in Table 1, the highest fluorescence intensity was obtained in methanol, therefore, it was used throughout this study. Fig. 1 shows the typical spectra of MKST in methanol.

Table 1: Effect of solvent on the fluorescence intensity of MKST (5 μg/ml)

Solvent	λ_{max} (nm)		Fluorescence intensity
	Excitation	Emission	
Methanol	340	390	90
0.1 M HCl	340	425	4
0.1 M NaOH	340	402	48
Water	340	465	24

Table 2: Effect of surfactants on the fluorescence intensity of MKST (5 μg/ml)

Surfactant	Fluorescence intensity
No surfactant	100.0%
Gelatin (100 μg/ml)	37.0%
Carboxymethylcellulose	88.4%
Sodium Dodecyl sulphate	92.2%
Triton X 100	110.8%
β-Cyclodextrin	115.1%

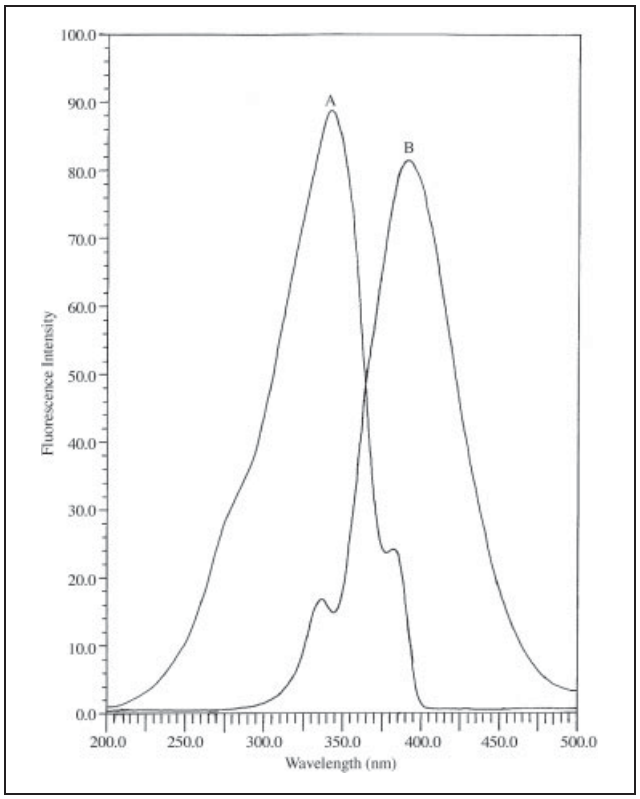


Fig. 1: Typical fluorescence spectra of montelukost sodium in methanol. A: Excitation spectrum; B: Emission spectrum

The effect of surfactants on the fluorescence intensity of MKST was studied by adding 1 ml (containing 100 μg/ml) of each surfactant solution to the drug solution in methanol. The results are abridged in Table 2. Gelatine caused a dramatic decrease in fluorescence intensity while carboxymethylcellulose and sodium dodecylsulphate caused a negligible decrease. Triton-X100 and β-cyclodextrin, on the other hand, caused a negligible increase in fluorescence intensity. Addition of large volumes of the surfactant aqueous solution, caused precipitation of MKST, therefore, no surfactants were used in this study. The relation between the fluorescence intensity and MKST sodium concentration is rectilinear over the range of 0.125 to 5.0 μg/ml. Linear regression analysis of the data gave the following equation:

$$RF = 1.375 + 16.04\,C \quad r = 0.9997 \qquad (1)$$

where RF is the relative fluorescence intensity and C is the concentration of MKST sodium in μg/ml.

The lower limit of detection was experimentally determined (S/N = 2) and was found to be 0.02 μg/ml (3.4×10^{-8} M). Statistical evaluation of the regression line, regarding standard deviation of the residuals ($S_{y/x}$), standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) gave the following values 0.73, 0.14 and 0.37, respectively. The small figures obtained point to the low scattering of the calibration points around the calibration line (Miller and Miller 1983).

To test the validity of the method, it was applied to the determination of an authentic sample of MKST sodium over the range 1–5 μg/ml (n = 7). The results are in good agreement with that given by the manufacturer (Table 3). The proposed method was then applied to the determination of MKST in tablets containing 4, 5 and 10 mg. The results obtained (Table 3) were in agreement with those given by the manufacturer adopting HPLC method. Common tablet excipients, such as talc, lactose, starch, avisil, gelatin, hydrogenated vegetable oils and magnesium stearate did not interfere with the assay.

The high sensitivity of the proposed method renders it applicable to the *in vitro* determination of MKST in biological fluids. MKST was successfully applied in spiked human plasma samples. The soluble proteins have to be precipitated by methanol followed by centrifugation. The fluorescence-concentration plot is rectilinear over the range of 0.4–2.0 μg/ml. Linear regression analysis of the data gave the following equation:

$$FI = 4.04 + 15.42\,C \quad R = 0.9992 \qquad (2)$$

where FI is the fluorescence intensity and C is the concentration in μg/ml.

The between-day variation was also studied by testing a sample of MKST concentration of 2 μg/ml on 4 successive days. The results are abridged in Table 4. The mean percentage recovery was 100.83 ± 1.53.

Table 3: Application of the proposed and reference methods to the analysis of MKST and its tablets

Form	% Recovery ± SD	
	Proposed method	Reference method
Pure form	100.53 ± 1.02	99.7 ± 0.8
Singulair Pediatric chewable tablets* (4 mg MKST/tablet). Batch # HS 89710	102.1 ± 3.36	99.5 ± 0.52
Singulair chewable tablets* (5 mg MKST/tablet) Batch # 224349	101.27 ± 2.30	99.0 ± 0.77
Singulair tablets* (10 mg MKST/tablet) Batch # 224385	101.07 ± 1.15	101.5 ± 0.72

* Products of Merck Sharp and Dohme, Hertford Road Hoddesdon, Hertforshire, UK

Table 4: Between day variation of recovery of MKST (2 μg/ml) from plasma using different standard curves

Day number	Correlation coefficient (r)	Amount found (μg/ml)	% Recovery
Day # 1	0.9994	2.065	103.25
Day # 2	0.9933	2.020	101.0
Day # 3	0.9987	1.987	99.35
Day # 4	0.9978	1.994	99.70
$\bar{X}$			100.83 ± 1.53

Table 5: Application of the proposed method to the determination of MKST in spiked human plasma

Amount added (μg/ml)	Amount found (μg/ml)	% Recovery
0.80	0.814	101.75
1.20	1.210	100.83
1.60	1.572	98.25
1.80	1.774	98.56
2.00	2.020	101.00
$\bar{X}$		100.08
SD		1.40

After establishing the proposed method, it was applied to the determination of spiked human plasma samples. The results are given in Table 5, and seem to be fairly accurate and precise. Fig. 2 shows the fluorescence spectra of spiked plasma upon addition of successive amounts of MKST.

Interference likely to be introduced from co-formulated drugs, such as loratadine or co-administered drugs, such as verapamil HCl, carbazepam, propranolol, or from common drugs, such as ascorbic acid, paracetamol, caffeine, glycine or cysteine were studied under the same experimental conditions using a methanolic solution containing 5 μg/ml. None of the studied drugs showed any interference at the wavelengths of MKST.

The effect of artificial daylight on the stability of MKST was studied by measuring the fluorescence intensity of a 3.0 μg/ml solution of MKST in methanol at different time intervals for 3 h. No significant decrease in fluorescence intensity was observed. No significant change also was observed upon exposing solution of the same concentration to light emitted from Deuterium lamp, the rate decrease of fluorescence intensity was negligible. On the other hand, upon exposing a solution of the same concentration to light from a Xenon arc lamp, it lost 50% of its fluorescence intensity in 25 min. A plot of log FI versus time is shown in Fig. 3. The decomposition is first-order. This behavior is due to the high energy content of the emitted light of the Xenon lamp. This necessitates that, the solution should be measured immediately and not left in the light path for more than a few seconds.

3. Experimental

3.1. Apparatus

The fluorescence intensities were measured using a spectrofluorimeter (Jasco, model FP 6200; Japan) equipped with a Xenon discharge lamp, excitation, emission gratting monochromators and a 1 cm quartz cell at low sensitivity. The apparatus was driven by a Pentium IV PC computer.

3.2. Reagents and materials

A reference standard sample of montelukast sodium (MK-0476, Batch # L-706, 631-001 M045) was generously provided by Merck Frost, Canada, Inc. (Quebec, Canada). Commercial tablets containing montelukast sodium were obtained from the Central Lab for Food and Drug Analysis, Riyadh, KSA: Singulair® tablets: each tablet contains 10 mg montelukast (Batch # 224385), Singulair® chewable tablets: each tablet contains 5 mg montelukast (Batch # 224349) and Singulair® paediatric tablets: each tablet contains 4 mg montelukast (Batch # 689710).

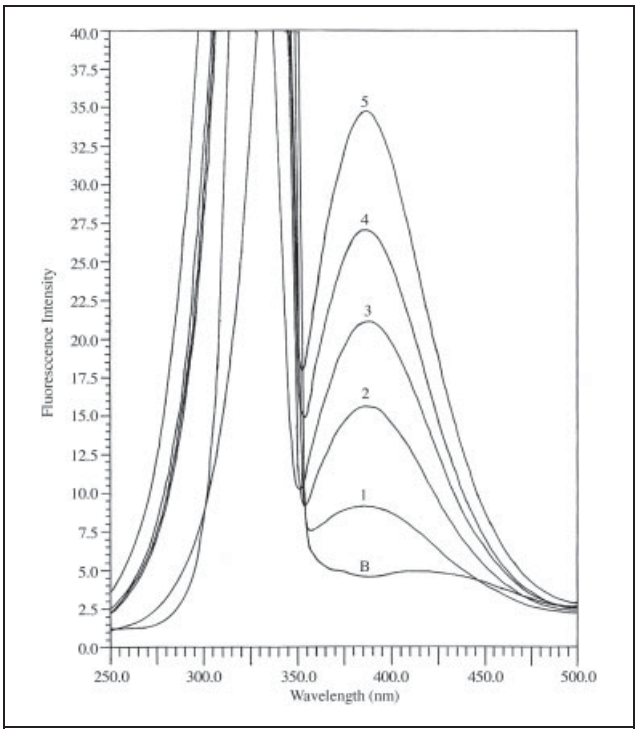


Fig. 2: Fluorescence spectra of plasma spiked with montelukast sodium. B: blank 1: 0.4 μg/ml, 2: 0.8 μg/ml, 3: 1.2 μg/ml, 4: 1.6 μg/ml, 5: 2.0 μg/ml

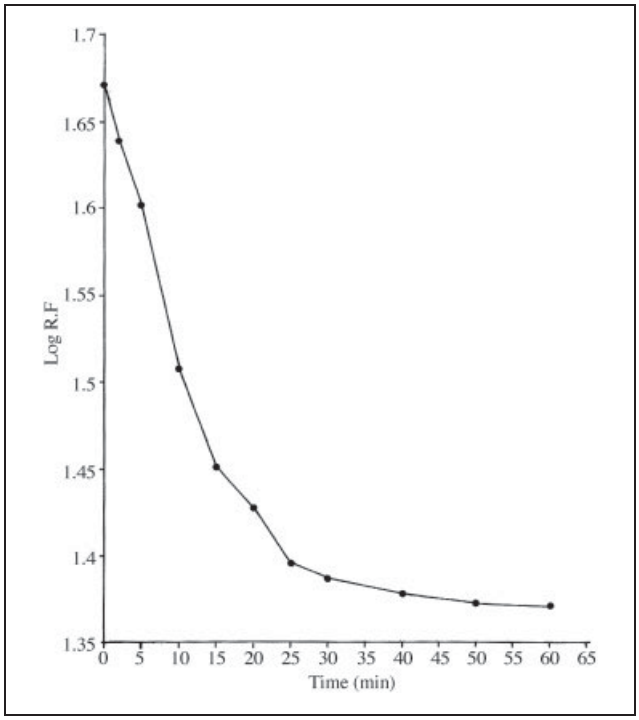


Fig. 3: Relation between log relative fluorescence (RF) and time of exposure to Zenon lamp

Plasma was obtained from King Khalid University Hospital, Riyadh, KSA, and was kept frozen until use after gentle thawing.
A stock solution of MKST sodium (10 mg/ml) in methanol was prepared. The solution was found to be stable for 4 days if kept in the refrigerator. Working standard solutions were further prepared by dilution with the same solvent as appropriate.

3.3. Calibration graph

Transfer aliquots of the working standard solution into a series of 25 ml volumetric flasks. Complete to the mark with methanol. Measure the fluorescence intensity at 390 nm after excitation at 340 nm. Plot the fluorescence intensity *versus* the final concentration to get the calibration graph. Alternatively, derive the corresponding regression equation.

3.4. Procedure for tablets

Weigh and pulverize 10 tablets. Transfer a weighed quantity of the powder equivalent to 10 mg of MKST sodium into a small flask. Add about 60 ml of methanol and sonicate for 30 min. Filter into a 100 ml volumetric flask. Wash the flask, residue and filter with methanol and add the washings to the same volumetric flask then complete to the mark with the same solvent. Proceed as described under 3.3. Determine the nominal content of the tablets using either the calibration graph or the regression equation.

3.5. Procedure for spiked human plasma

Transfer 1 ml aliquots of human plasma spiked with the drug into six 25-ml volumetric flasks. Complete to the mark with methanol and shake vigorously. Transfer portions of each flask into centrifugation tubes and centrifuge at 3000 rpm for 15 min. Measure the fluorescence of the clear supernatant at 340/390 nm. Plot the fluorescence intensities *versus* the final concentration to get the calibration graph. Alternatively, derive the corresponding regression equation.

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