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Flow injection analysis of meloxicam using UV-detection

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Meloxicam, (**1**,[4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is a non-steroidal anti-inflammatory drug of the oxicam class [1, 2]. A limited number of studies have been reported for the determination of **1** including first-derivative spectrophotometry and TLC densitometry [3], spectrophotometry and flow-injection was based on the formation of a green complex with Fe(III) [4] and HPLC [5].

The aim of this study was the direct determination of **1** by flow injection analysis (FIA) without colourizing the solutions and its application to the pharmaceutical preparations. To determine the parameters for the optimisation a **1** solution having 5×10^{-6} mol/l was used. The solvent system consisted of MeOH and bidistilled water. The percentage of MeOH ranged from 10% to 50% (v/v). It was found that the optimum concentration of MeOH, in view of peak morphology, was 10% (v/v). The best flow-rate was found to be 1 ml/min. The final concentration of buffer in the test solutions was 0.1 mol/l. When the base line was reached, another sample was injected. The peak areas versus pH was investigated. Because of the low solubility of **1** below pH 7 [6], significant differences were observed in peak areas. However, these differences are minimum at pH values between 8–10. Therefore, the buffer of pH was chosen as working pH. The signals of **1** at concentrations ranging from 1×10^{-6} to 5×10^{-6} mol/l were obtained under the conditions above and they are given in the Fig.

The relationship between the area under curve (AUC) and the concentration of **1** was found to be AUC = 1.28×10^{10}C(mol/l) + 26067.6; r = 0.9998. The detection limit (S/N = 3) was calculated to be 1.2×10^{-7} mol/l with RSD 1.23% (n = 8).

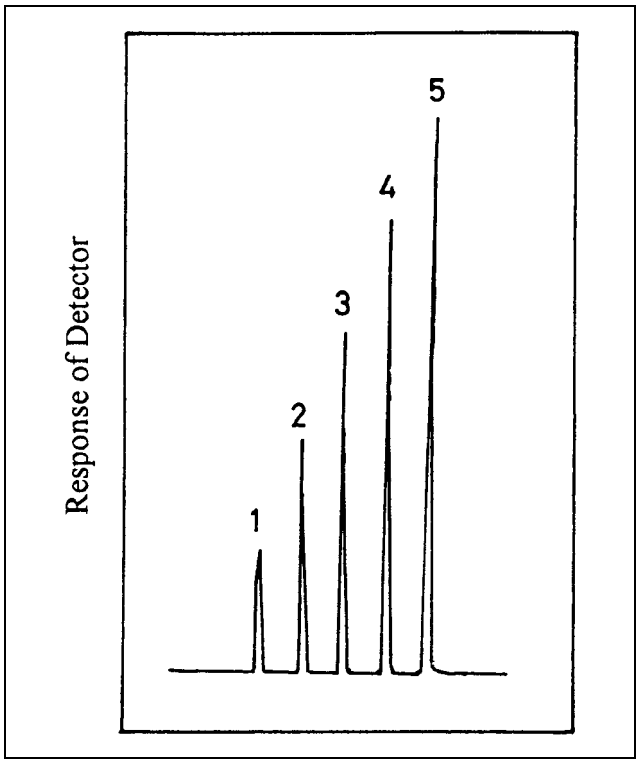

Fig: The signals in the 1×10^{-6}–5×10^{-6} mol/l concentration range of **1** at 360 nm

Table: Assay results of 1 in tablets*

	FIA	UV-Spectrophotometry
Mean (mg)	14.8	14.9
n	8	8
RSD%	1.3	0.8
CL	±0.15	±0.12
F-test of insignificant	2.38	$F_{0.05} = 4.28$ (table)
t-test of insignificant	1.35	$t_{0.05} = 2.18$ (table)

*Each tablet contains 15 mg of **1**

Linearity and accuracy were examined in the concentration range of 1×10^{-6}–5×10^{-6} mol/l employing intra-day and inter-day (for eight days) studies for the determination of **1.** Very accurate results were obtained for intra-day and inter-day experiments with a good correlation.

The validity of method was examined by applying to tablets. All results of the assays were evaluated statistically and presented in the Table. High reproducibility was observed and insignificant differences between FIA and UV-Spectrophotometry at the 95% probability level.

The method proposed in this study is accurate, precise and rapid. Therefore it can be suggested for the routine analysis of **1**.

Experimental

1. Apparatus and chemicals

The HPLC apparatus was a Model LC 6A pump equipped with a 20 µl manual loop injector, a Model SPD-A10 UV variable wavelength detector and a Model C-R7A integrator (all Shimadzu, Japan). Spectrophotometric studies were done using a Model UV-2401 PC (Shimadzu, Japan). A Model WTW Multiline P4 Universal pH-meter cabled Sen-Tix 92T pH electrode (Germany) was employed for measuring and adjusting the pH of the solution. Standard **1** (99.8%) and Melox® tablets containing 15 mg of **1** were kindly supplied from Nobel Ilaçları A.S. (Istanbul, Turkey). Standard **1** was used without further purification. Other chemicals were of analytical grade of Merck (Germany).

2. Procedures

2.1. Solutions

A stock solution of **1** (1×10^{-3} mol/l) was prepared using bidistilled water. Dilutions were made in the range of 1×10^{-6}–5×10^{-6} mol/l. As a mobile phase an aqueous solution of MeOH (10%, v/v) was used. The buffer solutions were prepared using 1 mol/l K_2HPO_4 (pH 7–11.9) and their pH values were adjusted using 2 mol/l HCl or 2 mol/l KOH.

2.2. Application to tablets

Ten tablets were weighed and finely powdered in a mortar. The average weight of a tablet was calculated. A sample equivalent to one tablet was weighed and transferred to a 100 ml calibrated flask, 1 ml phosphate buffer (1 mol/l, pH 9.3) and 10 ml MeOH was added, magnetically stirred for 20 min and made up to volume with bidistilled water. A sufficient amount of the solution was pipetted in a tube and was centrifuged for 10 min. The supernatant was diluted to the predetermined values and injected in to sample loop by means of a syringe. The absorbances was monitored at 360 nm. The area under curve values were used for calibration. UV-Spectrophotometry was chosen as a comparison method. The absorbances of the same solutions were measured at 360 nm using quartz cells. The relationship between absorbance (A) and concentration of **1** (C) was found to be A = 106970 C(mol/l) + 0.018; r = 0.9998.

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Received July 28, 2000
Accepted August 28, 2000

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The application of pig blood in the *in vitro* measurement of platelet adhesion

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Platelet adhesion to the damaged blood vessel is an essential step in the process of local hemostasis regulation. Different pharmaceuticals alter platelet susceptibility to physiological stimulation, therefore it is necessary to determine drug influence on platelet reactivity [1].
A method of measurement of human blood platelet adhesion to protein coated multiwell microplates has been previously agonists described by Bellavite et al. [2]. It is a simple and reproducible cell adhesion assay based on the determination of the acid phosphatase (PA) activity of platelets. As human blood is a precious material of restricted availability for research purposes, in this report the colorimetric assay of microtiter plate adhesion was applied using pig blood. Pig blood is easily available in substantial quantities, and its properties, as well as the similarities between human and pig morphological elements, enable the application of pig blood in *in vitro* assays [3, 4].
Our data demonstrated that the type of coating proteins used determined percentage of adhesion. The most sensitive stimulation of pig platelets with human Fb (Kabi) was observed. The results of PA activity in the case of both types of platelets (human and pig) are close, therefore the colorimetric assays of adhesion may be used in *in vitro* studies with platelets isolated from pig blood. In our opinion the assay described may be applied for screening purposes of new drugs.

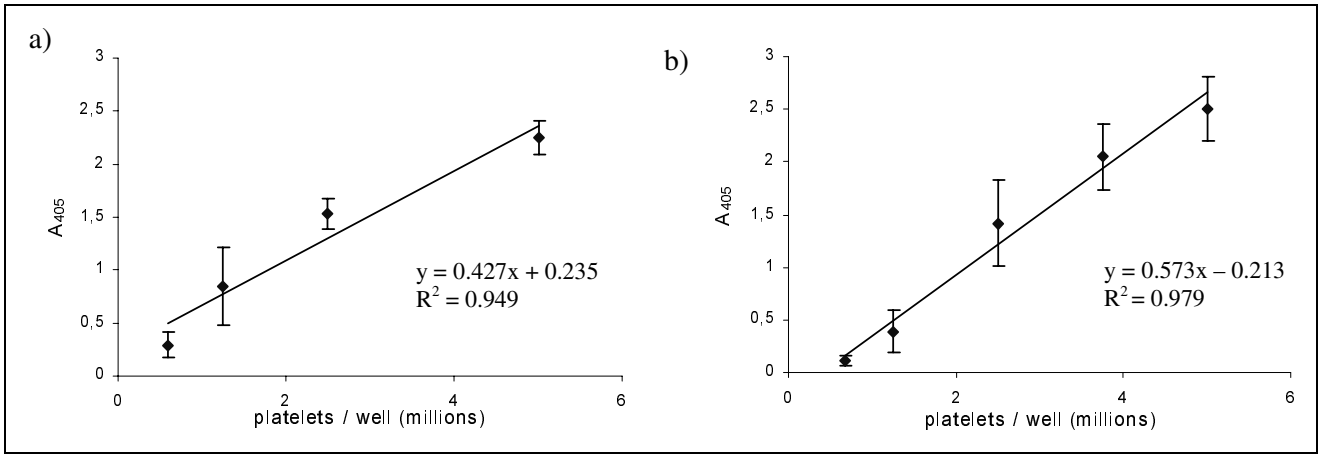

Fig.: Acid phosphatase activity as a function of a) pig and b) human platelet numbers (60 min incubation). Values are means ± SD (n = 3–11)

Table: Pig blood platelet adhesion to various proteins

	% Adhesion ± SD			
	Fibrinogen		Collagen	
	Human (Kabi)	Bovine (Serva)	Calf skin (Sigma)	Type I (Chrono – log)
Without ADP	15.4 ± 4.6 (n = 15)	17.1 ± 4.9 (n = 16)	16.9 ± 8.5 (n = 12)	16.0 ± 2.6 (n = 12)
With 10 µmol/l ADP (Sigma)	27.2 ± 4.6 (n = 15)	20.0 ± 5.2 (n = 20)	16.7 ± 8.3 (n = 11)	15.3 ± 2.2 (n = 12)