

RESEARCH ARTICLE

Spectroscopic and Colorimetric Determination of Meloxicam, Lornoxicam, Tenoxicam in Drugs

A. A. Chaplenko*, O. V. Monogarova, K. V. Oskolok

Department of Chemistry, Analytical Chemistry Division, Lomonosov Moscow State University, 119991 Moscow, Russia,

Received 12 Nov 2017; Revised 25 Dec 2017; Accepted 10 Jan 2018

ABSTRACT

New approach to spectrophotometric and colorimetric determination of meloxicam, lornoxicam, tenoxicam in drugs using 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD chloride) is developed. The techniques are based on alkaline hydrolyses of oxicams with NBD chloride and the subsequent spectrophotometric and colorimetric determination of the colored products of reaction. The linearity range of calibration dependence is 1-10 mg/ml for lornoxicam and tenoxicam, 0.5-5 mg/ml for meloxicam. The advantages of developed methods are rapidity and cost-efficiency. This approach can be used for screening control of drug quality.

Keywords: Meloxicam, lornoxicam, tenoxicam, screening of drug quality, spectroscopic determination.

INTRODUCTION

The development and optimization of new methods for the analysis and quality control of medicines is an actual applied problem of pharmaceutical and analytical chemistry. One of the modern approaches to analysis of drugs is the introduction of a preliminary quality assessment using express and inexpensive methods of analysis in the quality control procedure for medicines in a screening step^[1]. This approach allows to establish the inferiority of pharmaceutical preparations and to exclude them from circulation without a long and expensive procedure for complete standardization of drugs. Quality screening is advisable to apply to the most often falsified medicines, as well as pharmaceutical products, the quality of which falls particularly badly due to violations of the rules of production, transportation and storage. These drugs include non-steroidal anti-inflammatory drugs (NSAIDs). The main area of their application is the coping of pain syndrome and inflammatory reactions of various geneses^[2]. The safest modern groups of NSAIDs are selective inhibitors of cyclooxygenase-2 (COX-2), which practically do not have a side

effect on the gastrointestinal tract^[3]. Selected selective COX-2 inhibitors include some drugs of the oxicam group. Due to the presence of a chemically unstable amide group in the structure of oxicam molecules, even a slight deviation from the storage rules of the preparation, as well as a violation of the shelf life, can lead to a loss or decrease in their pharmacological activity^[4]. Some degradation products (e.g. 2-aminopyridine) have a toxic effect^[5]. Also control of the content of the main active substance, as preparations of the group of oxicams are among the most often falsifiable^[6]. Thus, the development of effective methods for controlling the quality of drugs of the group of oxicams for the purpose of determining the active substance and assessing stability is a very actual task.

To solve this problem it is expedient to use simple and accessible methods, one of which is digital colorimetry. This method is based on the analysis of a sample by mathematical processing of its digital image. A digital color analyzer can be any device that allows you to register a bitmap image and save it in a digital format, for example, a digital camera or photo scanner^[7]. The received digital information about color can be considered as an analytical one. Unlike visual colorimetry, which is usually used for semiquantitative determination, digital color analysis allows to

*Corresponding Author:

A. A. Chaplenko,

Email: a.a.chaplenko@yandex.ru

obtain more accurate data. The main advantages of colorimetry are the speed, simplicity and low cost of analysis^[8].

7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD chloride) is a reagent traditionally used in biochemistry as a fluorescent label for compounds containing amino groups (especially peptides and proteins) because it interacts with them intensively stained and fluorescent compounds. NBD chloride was first introduced as a reagent for the fluorimetric detection of amines in 1968 and has long been used exclusively for the determination of proteins and peptides after chromatographic separation^[9]. More recently, techniques have been developed for spectrophotometric and spectrofluorimetric determination of some antihistamines and antihypertensive drugs based on the products of their interaction with NBD chloride^[10]. The techniques are express and have high sensitivity, accuracy of the analysis.

The purpose of this research is the development of techniques for spectrophotometric and colorimetric determination of meloxicam, lornoxicam and tenoxicam in drugs based on the products of alkaline hydrolysis of oxicams with NBD chloride.

MATERIALS AND METHODS

Meloxicam medication (Mawalis®, intramuscular injection 10 mg/mL, ampoule 1.5 mL, Amelotex®, intramuscular injection 10 mg/mL, ampoule 1.5 mL, Artrozan®, intramuscular injection 10 mg/mL, ampoule 1.5 mL), Lornoxicam (Xefocam®, lyophilizate for the preparation of a solution for intravenous and intramuscular administration of 8 mg complete with a 2 mL solvent), Tenoxicam (Texamen®, lyophilizate for the preparation of a solution for intravenous and intramuscular administration of 20 mg, a vial of solvent (water for injection) 5 mL) are used in the work.

For the preparation of calibration samples, drugs analyzed according to the procedures given in the normative documentation^[11], in an accredited expert laboratory were used (in parentheses indicates the found content of the active substance relative to the declared by the manufacturer) – Mawalis® (100.1 ± 0.3%), Amelotex® (99.7 ± 0.4%), Artrozan® (99.9 ± 0.6%), Xefocam® (99.4 ± 0.9%), Texamen® (100.1 ± 0.5 %).

EXPERIMENTAL

Alkaline decomposition of oxicams

1 mL of the contents of the ampoules of Meloxicam drug or 2 mL of reconstituted lyophilizate of Lornoxicam and Tenoxicam drugs were quantitatively transferred to volumetric flasks with a capacity of 20.0 mL and the volume was adjusted to the mark with 2M solution NaOH, was heated in a water bath with vigorous stirring for 20 minutes. Then the mixture with the products of alkaline degradation of oxicams was cooled, quantitatively transferred to volumetric flasks (100.0 mL) and distilled water was added up to the mark. The resulting solutions were used for preparation of calibration samples.

Photometry reaction

An aliquot of solutions of alkaline degradation products of oxicam (1.00 mL) was transferred to a conical flask, 1 mL of 0.5 % solution of NBD chloride (excess), 1 mL of borate buffer (pH 8.0) and heated in a water bath for 30 minutes, after which the solution with the colored reaction product was quantitatively transferred to a volumetric flask, and methanol was added to 10.0 mL. Blank solutions were prepared in a manner analogous to the analyte solutions of the drugs, excluding the alkaline step.

The measurement of analytical signal

The absorption spectra of the analyzed and calibration solutions were measured on a Shimadzu UV-1601 spectrophotometer (Japan) at a wavelength of 461 nm corresponding to the absorption maximum of the product of the photometric reaction. The determination of the oxicam content was carried out by a calibration schedule method. The cuvettes with analyzed and blank solutions were placed in the center of the box of 300-3300-3300 mm corrugated cardboard with a white interior finish, after which the cuvette with the sample was photographed; an image was obtained in the jpg-format (size 1920 ´ 1080 pixels). As an analytical signal, the mean value of the R-channel lightness of the digital image region of a cuvette of 300 ´ 300 pixels size was used.

RESULTS AND DISCUSSION

NBD chloride reacts with primary aromatic amines by the mechanism of electrophilic substitution. In contrast to oxicams, the products of alkaline hydrolysis (2-aminopyridine, 2-methylthiazole) contain a primary amino group. These primary aromatic amines react with NBD chloride to form a tan compound having fluorescence. Thus, the reaction of hydrolyzate with NBD chloride can be used for spectrophotometric or spectrofluorimetric determination of the impurity of the products of oxicams decomposition in drugs, the determination of active substances, and also for the evaluation of

the stability of oxicam preparations. In this paper, we propose an approach based on the alkaline hydrolysis of the active substances of meloxicam, tenoxicam, lornoxicam, followed by interaction of the reaction product with NBD chloride in borate buffer medium and measuring the optical density of the resulting colored solution.

At the first stage, the amide bond is hydrolyzed in oxicam molecules. It has been established that heating in a water bath in 2M NaOH for 30 min leads to complete hydrolysis of the oxicams (Fig. 1) to form 2-aminopyridine (2-AP) (lornoxicam and tenoxicam) or 2-aminomethylthiazole (2-AMT) (meloxicam) (Fig. 2). A colored and fluorescent compound is formed by the interaction of 2-AP or 2-AMT with NBD chloride (Fig. 3). It was shown experimentally that the maximum of optical density of colored products for oxicams is observed at the same wavelength of 461 nm. Since the hydrolysis can occur naturally during the storage of the drug, it is important to use a solution of the drug substance that has not undergone additional alkaline hydrolysis after interaction with NBD chloride as a reference solution. It was shown that intact samples of drugs do not form colored compounds when interacting with NBD chloride, because the reference solution does not absorb

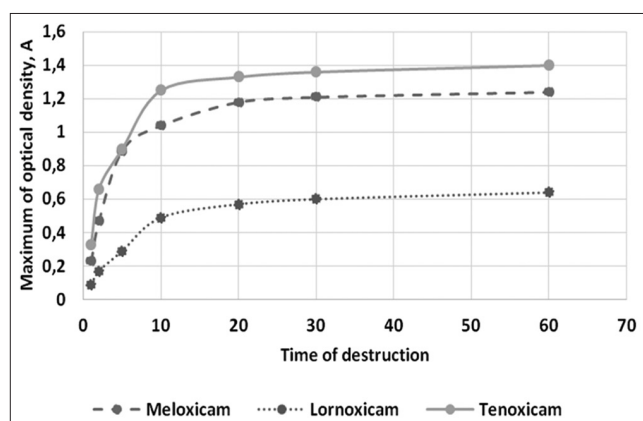


Figure 1: The determination of optimal time for alkaline hydrolysis of oxicams

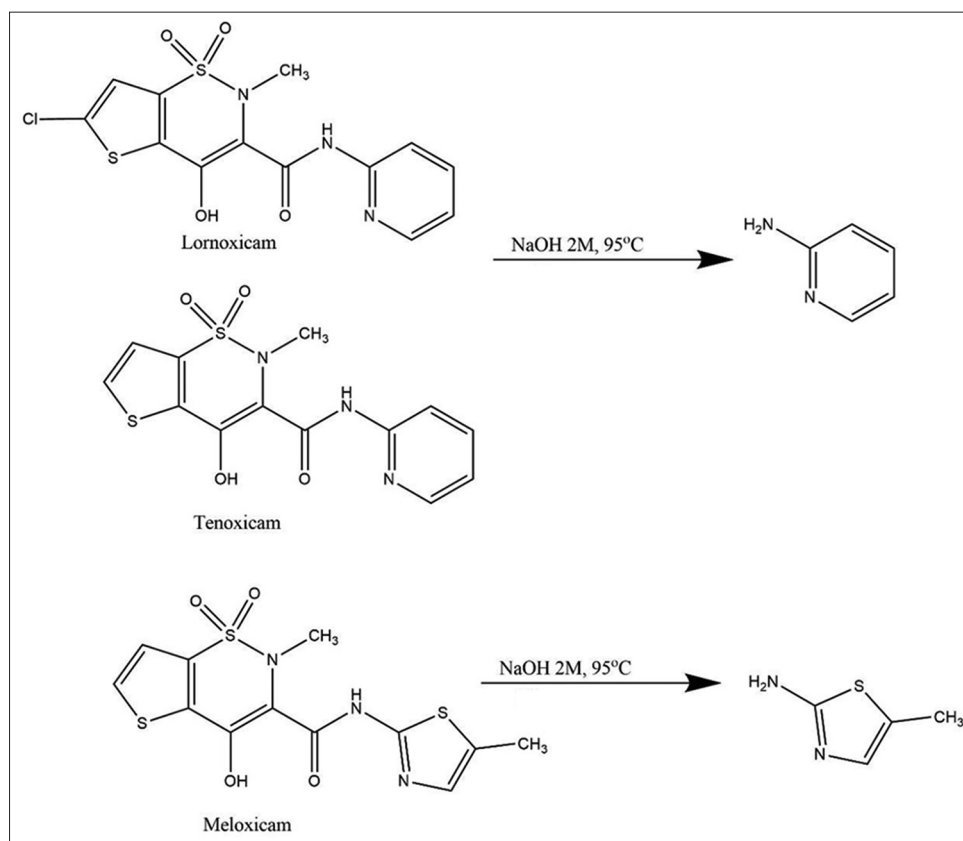


Figure 2: Products of alkaline hydrolysis of meloxicam, lornoxicam and tenoxicam^[12].

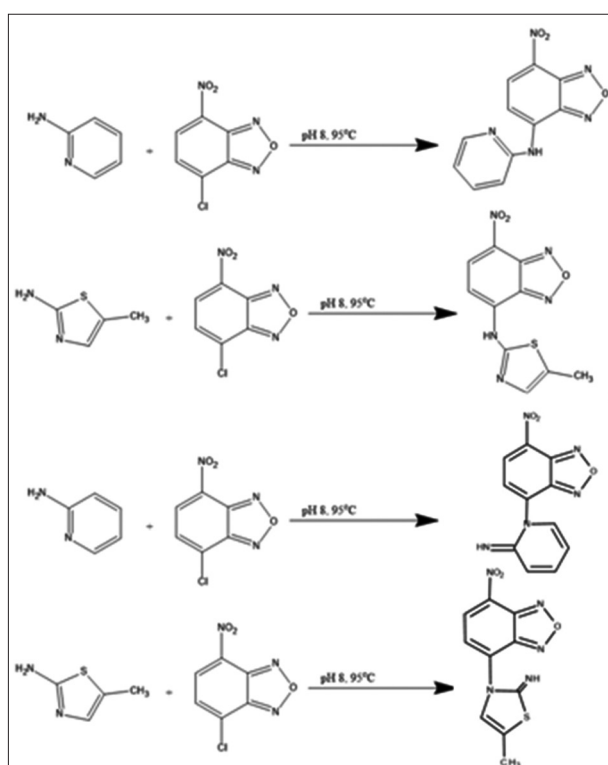
Table 1: Metrological features of developed techniques. $P=0.95$, $n=10$.

	Meloxicam		Lornoxicam		Tenoxicam	
	SM*	CM**	SM	CM	SM	CM
Linearity range, $\mu\text{g/ml}$	0.5-5	0.5-5	1-10	1-10	1-10	1-10
Mass absorption coefficient, $\%^{-1} \cdot \text{cm}^{-1}$	1489	-	1687	-	1821	-
Standard deviation (δ), $\%^{-1}$	6	14	4	16	5	18
Relative standard deviation (sr), %	4	18	6	14	5	11

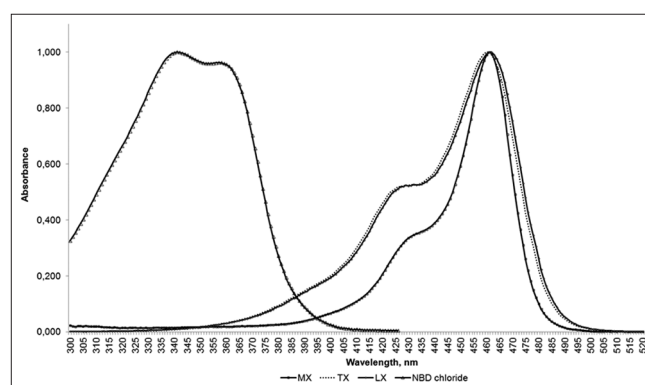
* spectrophotometry, ** colorimetry

Table 2: The results of spectrophotometric and colorimetric determination of oxicams in drugs. $P=0.95$, $n=10$.

Drug	Nominal content of active substance per vial, mg	Founded by	
		spectrophotometry	colorimetry
Amelotex [®]	15.0	14.7 \pm 0.3	14.5 \pm 0.9
Xefocam [®]	8.0	8.1 \pm 0.1	8.1 \pm 0.7
Texamen [®]	2.0	19.3 \pm 0.3	19.3 \pm 0.7

**Figure 3:** Reactions of the products of hydrolysis of meloxicam, lornoxicam and tenoxicam with NBD-chloride, proceeding with the formation of colored compounds

($A < 0.05$ relative to bidistillate) in the region of 461 ± 5 nm (absorption maximum of NBD chloride is 337 nm) (Fig. 4). Thus, the developed approach can be used to assess the stability of drugs. Alkaline hydrolysis of the amide bond at room temperature is very slow, so the reaction was carried out with heating in the water bath, the optimum heating time was determined experimentally (Fig. 1). Other conditions of the analysis (the choice of methanol as a solvent for spectrophotometry, temperature, pH and the time for the interaction of NBD chloride with the hydrolysis products)

**Figure 4:** UV/Vis spectra of NBD-chloride and coloured compounds, proceeded from meloxicam, lornoxicam and tenoxicam

were selected according to the data of the study^[10]. Metrological features of the developed techniques are presented in the Table 1. Results of analysis of real drugs are presented in the Table 2.

CONCLUSIONS

The techniques of spectrophotometric and colorimetric determination of active substances in drugs of the oxicam groups have been developed. The main advantages of using the spectrophotometric and colorimetric approaches are the rapidity, low cost and high efficiency, as well as the possibility of using the techniques for assessing the quantitative content of active substances, the purity and stability of the drugs.

REFERENCES

- Hetrick E. M., Vannoy J., Montgomery L. L., Pack B. W. Integrating tristimulus colorimetry into pharmaceutical

- development for color selection and physical appearance control: A quality-by-design approach. *Journal of pharmaceutical sciences*. 2013. 102 (8). 2608-2621.
- Graham G. G., Davies M. J., Day R. O., Mohamudally A., Scott K. F. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology*. 2013. 21(3). 201-232.
 - Patrignani P., Patrono C. Cyclooxygenase inhibitors: from pharmacology to clinical read-outs *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2015. 1851(4). 422-432.
 - Starek M., Dąbrowska M., Bracha M., Opoka W. Experimental study of the stability of some oxicams in contact with various redox agents. *JPC-Journal of Planar Chromatography-Modern TLC*. 2016. 29(4). 273-280.
 - Mayorov A. V., Willis B., Di Mola A. et al. Symptomatic relief of botulinum neurotoxin/a intoxication with aminopyridines: a new twist on an old molecule *ACS chemical biology*. 2010. 5(12). 1183-1191.
 - Rebiere H., Guinot P., Chauvey D., Brenier, C. Fighting falsified medicines: The analytical approach. *Journal of Pharmaceutical and Biomedical Analysis*. 2017. 142. 286-306
 - Philp M., Fu S. A review of chemical 'spot' tests: a presumptive illicit drug identification technique. *Drug testing and analysis*. 2017. 1-14.
 - Kumpanenko I. V., Roshchin A. V., Ivanova N. A., Bloshenko A. V., Shalynina N. A., Korneeva T. N. Colorimetry: Choice of colorimetric parameters for chromophore concentration measurements. *Russian Journal of General Chemistry*. 2014. 84 (11). 2295-2304.
 - Ghosh P. B., Whitehouse M. W. 7-chloro-4-nitrobenz-2-oxa-1,3-diazole: a new fluorogenic reagent for amino acids and other amines. *Biochemical journal*. 1968. 108 (1). 155.
 - Abd El-Hay S. S., Colyer C. L., Hassan W. S., Shalaby A. Utility of 7-chloro-4-nitrobenzofurazan (NBD-Cl) for the spectrophotometric and spectrofluorimetric determination of several antihistamine antihypertensive drugs. *Journal of AOAC International*. 2013. 96(5). 968.
 - United States Pharmacopeia 41 – National Formulary 36.
 - Taha E. A., Salama N. N., Fittakh L. E. Spectrofluorimetric and spectrophotometric stability-indicating methods for determination of some oxicams using 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl). *Chemical and pharmaceutical bulletin*. 2006. 54(5). 653.