Investigation of the pharmacological effects and analytical methods of Meclofenoxate and its metabolites

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Abstract. This article provides a review and outlook on the current status and development of AICAR in the fields of medicine and anti-doping.Meclofenoxate, as a central stimulant derived from phenoxyacetic acid ester, can enhance the activity of inhibited nervous system and modulate the metabolism of nerve cells. Its misuse among athletes places it on the international list of banned stimulants, posing a significant threat to the health of athletes and the integrity of sports. Hence, this paper summarizes the current research status on the pharmacological effects and analytical methods of meclofenoxate, aiming to serve as a reference for future research on this compound.

Keywords: Meclofenoxate; Doping; Pharmacological effects; Assay.

1. Introduction

Meclofenoxate is a central nervous excitatory drug, its chemical name is dimethylaminoethyl p-chlorophenoxyacetate, the structural formula is shown in Figure 1, mainly acts on the cerebral cortex, it can promote the oxidation reduction of brain cells, regulate the metabolism of nerve cells, increase the use of sugar, and have an excitatory effect on the inhibited central nervous system. It is mostly used clinically for traumatic coma, neonatal hypoxia, pediatric enuresis, disorders of consciousness, cognitive dysfunction and senile dementia and alcohol poisoning, carbon monoxide poisoning, etc [1-3]. Meclofenoxate is also an intellectual stimulant and can be used as a dietary supplement. China has been producing this drug since the 1960s, meclofenoxate raw materials are cheap and easy to obtain, the production process is simple, the market share is high [4], because it has the characteristics of effective stimulation of mental excitement, inhibit fatigue, etc. With the rapid development of competitive sport, the complexity of human metabolism and the increasing level of doping by athletes, in-competition abuse of meclofenoxate by athletes has become increasingly common. This effect allows athletes to improve their performance by this means, not only a serious violation of the spirit of competitive sports, but also may cause serious physical damage to athletes, such as the development of drug dependence, the development of serious personality changes, leading to cellular and carbon dioxide poisoning, The main hazards include the development of serious personality changes, abnormal cell and organ functions, allergic reactions, and compromised immunity. For this reason, the World Anti-Doping Agency (WADA) has classified meclofenamate as an S6 In-Competition Prohibited Substance in the field of sport[5].

Meclofenoxate is rapidly degraded to 4-chlorophenoxyacetic acid (4-CPA) in biological fluids (e.g. human plasma or urine) [6], but the presence of 4-CPA in urine may result not only from the use of meclofenoxate but also from the following permitted dosing: (1) foods containing 4-CPA residues, which are also used as herbicides and plant growth regulators in some countries or regions of the world [7-9] ; (2) chlorphenesin [3- (p-chlorophenoxy)-propane-1,2-diol], a non-prohibited substance used as a preservative in cosmetics and lotions[10]or approved for use in specific countries, such as chlorfenvinyl carbamate, a centrally acting skeletal muscle relaxant used to relieve muscle pain that can be converted to 4-CPA in vivo and therefore also represents a potential source of target analytes to be considered in anti-doping testing procedures to be considered in anti-doping testing procedures[11].

At present, there are few studies on the detection and analysis of meclofenoxate at home and abroad, which brings certain challenges to the actual detection and regulation. In view of this, this paper provides an overview of the pharmacological effects and pretreatment methods of meclofenoxate, focuses on the research progress of meclofenoxate detection methods, and provides an outlook on the problems it faces.

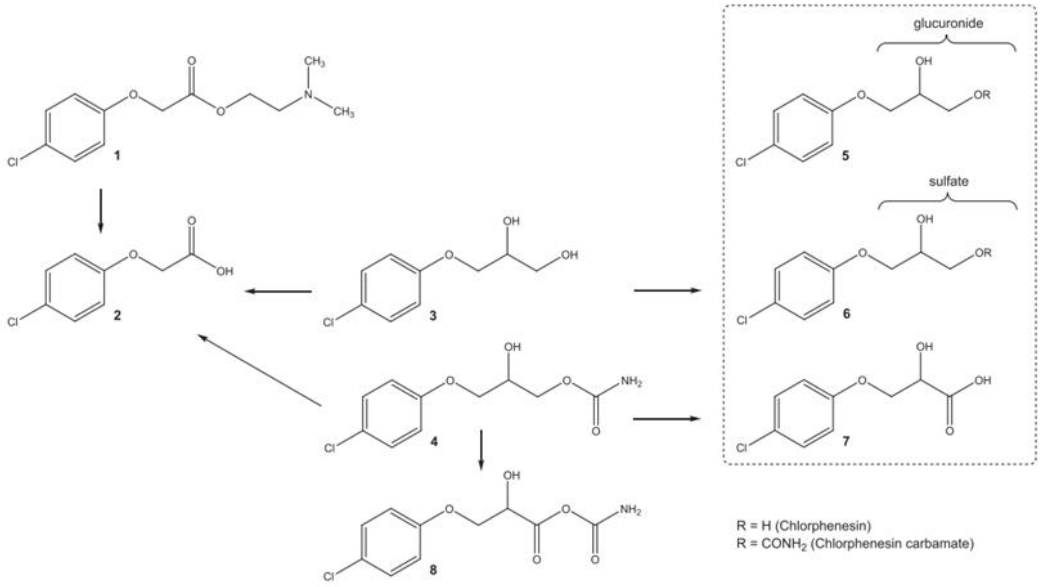

Fig.1 Chemical structures of meclofenoxate (1), 4-chlorophenoxy acetic acid (4-CPA, 2), chlorphenesin (3), chlorphenesin carbamate (4), chlorphenesin glucuronide (5), chlorphenesin sulfate (6), 3-(4-chlorophenoxy)-2-hydroxypropanoic acid (4-CPP, 7), and 4-CPP carbamate (8).

2. Pharmacological effects

2.1 Oxygen radical scavenging effect

BStudies have shown that meclofenoxate has potent free radical scavenging effects, particularly on hydroxyl radicals. By scavenging hydroxyl radicals and reducing protein cross-linking, meclofenoxate significantly reversed the changes in insoluble protein levels with age in the brain and liver of mice. In addition, meclofenoxate increased the activity of antioxidant enzymes including superoxide dismutase and glutathione peroxidase in vivo, and increased the body's oxygen radical scavenging capacity. Some studies have shown that oxidative stress is an important factor in the development of Alzheimer's disease, and clinical trials have reported that meclofenoxate has the potential to be a therapeutic agent for this disease[12].

2.2 Removal of intracellular lipofuscin

Lipofuscin is a brownish-yellow particle that accumulates in the body with age and has autofluorescent properties. When malondialdehyde, the end product of lipid peroxidation metabolism, combines with phosphatidylethanolamine to produce a fluorochrome, it then binds to proteins, peptides and lipids to form lamellar lipofuscin. Although lipofuscin itself is not toxic, it accumulates in the body and occupies some space in the cell, thus disrupting the normal metabolic transport function of the cell. Animal experiments have shown that meclofenoxate significantly reduces the deposition of lipofuscin in brain cells of different brain regions by a mechanism related to the inhibition of lipid peroxidation, the strength of the effect depends mainly on the duration of administration, and the reduction of lipofuscin deposition becomes more pronounced with longer administration time, while the function of neurons is also significantly improved [12].

2.3 Promotion of brain metabolism

Meclofenoxate can stimulate glucose uptake, increase oxygen consumption and carbon dioxide production, inhibit glucose enzymes, promote pentose phosphate pathway, and promote energy metabolism in brain cells by regulating the activities of key enzymes in the tricarboxylic acid cycle, pentose phosphate pathway and mitochondrial electron transport chain such as hexokinase, phosphofructokinase, malate dehydrogenase, glucose 6-phosphate dehydrogenase, and cytochrome C reductase. etc[13].

2.4 Effects on neurotransmitters in the central nervous system

Meclofenoxate affects the levels of various neurotransmitters in the CNS and their affinities with the corresponding receptors. Meclofenamate significantly increased acetylcholine levels in the CNS, increased the affinity of M receptors for acetylcholine, and recorded a significant increase in the corresponding electrophysiological activity, with a parallel trend, and the increase in electrophysiological activity could last longer. This mechanism may be related to the pro-intellectual effects of meclofenamate and improved cognitive function. Monoamine neurotransmitters include dopamine, norepinephrine and 5-hydroxytryptamine, whose functions involve their biosynthesis, storage, release and reabsorption. The application of meclofenamate reduces the level of norepinephrine and increases the level of dopamine and 5-hydroxytryptamine in the central nervous system. These effects may be one of the pharmacological bases for the availability of meclofenoxate for the treatment of Alzheimer's disease[14].

2.5 Protective effect on cerebral ischemia and hypoxia

The results of experimental study showed[14]that meclofenoxate could improve the resistance of experimental animals to various types of cerebral ischemia and hypoxia, and significantly increase the blood flow to cerebral regions such as cerebral cortex, thalamus and basal ganglia. Applying to the rat focal cerebral ischemia model, meclofenoxate reduced the area of cerebral infarction, improved neuronal ultrastructure, and had a significant anti-cerebral ischemic-hypoxic effect, which may be related to the anti-oxidative free radical effect of meclofenoxate hydrochloride, long-term administration to promote cell membrane lecithin synthesis, and regulation of phospholipid metabolism.

2.6 Other effects

Meclofenoxate also has some other common pharmacological effects, such as improving the learning ability and memory of the brain[13], which may be related to the influence of meclofenoxate on the level of monoamine neurotransmitters in the brain; meclofenoxate can activate the function of the brainstem upstream reticular formation system, increase the unit discharge of the reticular formation, and promote the awakening of the role of the brain; and also increase the synthesis of choline and phospholipids, so as to achieve the effect of the protection of the biofilm[14], and so on.

3. Biological Sample analysis

3.1 Biological sample pre-treatment

Most of the studies for the detection of meclofenoxate and other related substances in biological samples have focused on test materials such as blood and urine, where the main pre-treatment methods are liquid-liquid extraction, solid-phase extraction and protein precipitation. In recent years, the analysis of natural urine samples after direct injection offers a promising analytical method [15]with broad applicability to many different compounds and their metabolites, eliminating the need for time-consuming sample preparation processes while reducing costs and facilitating the

expansion of the analytical capacity of laboratory doping assays[16]. Table 1 summarizes the pre-treatment methods available in the literature.

Table 1 Pre-treatment methods for meclofenoxate and metabolites

Sample	Processing method	Main steps	Recovery rate	References
Blood	Protein precipitation method	Acetonitrile and protein are adde for precipitation	88.1%～90.5%	[15]
Blood	Liquid-liquid extraction	Adding 0.5 mol·L^{-1}sodium hydroxide solution to a djust to alkaline, adding ethyl acetate for extraction after uniform mixing, and then adding 0.1% formi c acid aqueous solution for vortex back extraction	98.7～101.9%	[20]
Blood	Solid phase extraction	Methanol-water (1:1,V/V) was added, followed by ammonia-ammonium chloride buffer (pH 11) and n-hexane-dichloromethane-isopropanol (300:150:1 5,V/V) was added	77.2%～84%	[21]
Urine	Direct injection method	Add 10 μL of 50% methanol to 90 μL urine at 1 μg/mL and inject 5 μL directly into the LC-HRMS/MS instrument	——	[22]

Currently, the methods used for the detection of meclofenoxate and its metabolites include high performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS), electrochemical methods, etc. The HPLC method is simpler and faster, but less sensitive than the LC-MS method, and the LC-MS detector with strong ion screening ability and high selective specificity is the most commonly used analytical method in laboratories, but the instruments are expensive. Table 2 summarizes the detection methods related to meclofenoxate and its metabolites.

Table 2 Assays for meclofenoxate and its metabolites

Target	Material inspection	Test method	Linearity range	Detection limit	References
4-chlorophenoxyacetic 5- acid (4-CPA)	Urine	LC-MS	——	——	[24]
Methyl chloride hydrochloride Fenyl ester	Meclofenoxate hydrochloride disket	HPLC	5.2～20.8 μg/mL	2 ng/mL	[25]
Meclofenoxate hydrochloride	Blood plasma	Electrochemical luminescence	0.01～50 μg/mL	6.3 ng/mL	[26]
Meclofenoxate	Meclofenoxate hydrochloride	Nuclear magnetic resonance quantitative method	——	——	[27]
Meclofenoxate hydrochloride	Meclofenoxate hydrochloride preparation	Spectrophotometri c method	2～18 μg/mL	——	[28]

3.2 High performance liquid chromatography

High performance liquid chromatography (HPLC) is widely used in the separation and identification of drugs. HPLC is mostly used for quality control studies in the clinical use of meclofenoxate hydrochloride capsules and dispersible tablets. Zhou et al. [22]established a high performance liquid chromatographic method for the determination of the content and related substances of meclofenoxate hydrochloride dispersible tablets. The results showed that the product was degraded under acid, alkali, oxidation, strong light and high temperature conditions with different degrees of damage, and the degradation products could be effectively separated from the main peak of the sample, and the separation was in accordance with the requirements, the minimum detection amount was 2.0 ng, the average content of related substances in three batches of samples

was 1.56%, and the sample The sample concentration showed a good linear relationship with the peak area in the range of 5.2-20.8 μg/mL, and the average recovery was 99.26%, which has the advantages of reliable results and good reproducibility for the quality control of meclofenoxate hydrochloride dispersible tablets. Meng Ting et al[26]. used high performance liquid chromatography (HPLC) with a column of YWGC18, methanol-tris-ethylamine phosphate buffer (pH 3.2) in a volume ratio of 45:55 as the mobile phase, and the detection wavelength was 228 nm to achieve the determination of p-chlorophenoxyacetic acid, a synthetic intermediate of meclofenoxate for injection, with the detection limit of 0.042 mg/L. Wang et al. [28]used this method to investigate the effects of organic phase ratio and The method is sensitive, specific, easy to operate, and solves the problem of imperfect chromatographic conditions of the existing standard for the determination of the substance of meclofenoxate hydrochloride, and provides data reference for the revision of this series of quality standards. It also provides a strong technical support for the scientific supervision of drugs and the consistency evaluation of generic drug quality of injectables.

3.3 Liquid chromatography-mass spectrometry (LC-MS)

With the characteristics of accurate qualitative and quantitative analysis and wide range of applications, LC-MS method is gradually becoming a mainstream instrument in the analytical industry and is suitable for the study of the detection of substances such as meclofenoxate. Nan et al. [17]established a method for the determination of dimethylaminoethanol (DMAE), the active metabolite of meclofenoxate, in human plasma by high performance liquid chromatography-mass spectrometry (HPLC-MS) with MRM mode detection and external standard method for quantification, the intra-day and inter-day precision was less than 4.8% and 6.0%, respectively, and the extraction recoveries were 32.5%~34.5%, indicating that the method is suitable for the determination of DMAE concentration in plasma and pharmacokinetic and bioavailability studies.

High-resolution mass spectrometry has higher resolution and can be used for screening of unknowns, structural resolution of compounds, and high-throughput detection using accurate mass numbers. Current detection of banned substances in sports is mainly based on urine analysis, focusing in many cases on the characteristic metabolites of the drug[18]. For the banned stimulant meclofenoxate, the main target analyte is 4-chlorophenoxyacetic acid (4-CPA), in order to detect the presence of metabolites of these substances in the sample and thus provide evidence for the origin of 4-CPA, as in Rubio et al[21].. In order to differentiate between chlorphenesin, 4-CPA produced by chlorphenesin carbamate and meclofenoxate, the urinary metabolite profile of sunscreen containing chlorphenesin was investigated, and urine samples were prepared by direct injection method and resolved by liquid chromatography-high resolution mass spectrometry detection, with the mobile phase being a mixture of 5 mM ammonium acetate and acetonitrile containing 0.1% acetic acid and quantified by internal standard method. Accurate mass analysis of the parent and characteristic product ions of the substance to identify urinary metabolites according to the literature showed that 4-CPA is a common metabolite of meclofenoxate, chlorphenesin and chloroformate, and that monitoring diagnostic urinary metabolites of chlorphenesin may provide conclusive supporting evidence for the use of chlorphenesin or prohibited meclofenoxate. Krug et al. [23] measured the excretion of nitrogen oxides from 2-(Dimethylamino)ethan-1-ol (deanol), another urinary metabolite of meclofenoxate, by LC-MS technique and compared it with the concentration levels and elimination of deanol-N-oxides following subject dosing of deanol, and showed that the use of deanol and meclofenoxate led to a significant elevation in the urinary deanol-N-oxide levels. In sports drug testing, different screening procedures need to be used for different classes of compounds, and with the growing list of doping prohibited substances, it is important to reduce sample preparation procedures. Goergens et al[15]. proposed a multi-target assay based on liquid chromatography-high resolution mass spectrometry with sample processing using a dilution-injection assay for screening compounds including meclofenoxate. The method is based on liquid chromatographyhigh resolution mass spectrometry. The HPLC and LC-MS assays for meclofenamate and metabolites are summarized in Table 3.

Table 3 HPLC and LC-MS methods for the determination of meclofenoxate and its metabolites

Target	Detecting instrument	Chromatographic condition		Mass spectral condition		Linearity range	References
		Mobile phase	Chromatographic column	Ion pair (m/z)	Ion source detection mode		
Meclofenoxate hydrochloride	Liquid chromatography-high resolution mass spectrometry	5 mM ammonium acetate buffer containing 0.1% acetic acid and acetonitrile	C18	256.0746、258.0716	Negative ion mode	——	[14]
dimethylaminoethanol	Liquid chromatography-mass spectrometry/mass spectrometry	Methanol-water (80:20) (containing 0.1% formic acid)	C18	90.1、72.1	Positive ion mode	3.125～500 ng/mL	[21]
Meclofenoxate hydrochloride	high efficiency liquid chromatography	V(Acetonitrile): V(0.12% NH_4HCO_3-0.5% Triethylamine) solution=33:67(methanesulfonic acid adjusted to pH3.0)	C18	——	——	1.632～163.2 μg/mL	[29]
Meclofenoxate hydrochloride	high efficiency liquid chromatography	Phosphate Triethylamine Buffer-Acetonitrile (65:35,V:V)	C18	——	——	8～70 μg/mL	[30]

3.4 Electrochemical methods

The expanded application of portable analytical devices can be attributed to the development of efficient and stable chemical sensors suitable for the determination of trace substances in simple matrices. Khalil et al. [31]Construction and sensing of carbon nanotubes and TiO2 nanotubes based carbon paste sensors for trace determination of meclofenoxate hydrochloride and discussing the role of lipophilic anionic additives and plasticizers, the constructed potentiometric sensors have been successfully used in pharmaceuticals, The constructed potentiometric sensors have been successfully used for drug determination in pharmaceuticals, spiked surface water and human urine samples, and the detection limits of carbon nanotube CPE and TiO2 -CPE were 7.6×10-7, 6.5×10-7 mol/L and the recoveries were 5.99 and 8.2%, respectively. Hua et al.[24] established an indirect method for the determination of meclofenoxate hydrochloride in human plasma by using capillary electrophoresis with electrochemiluminescence detection, and the hydrolysis product of meclofenoxate significantly improved. The results showed that the recovery of meclofenoxate ranged from 95.9 % to 96.9 % under optimal conditions, and the method can be successfully applied to the determination of meclofenoxate hydrochloride (MFX) in human plasma. Aleem et al. [32]developed a disposable screen-printed sensor method for the determination of meclofenoxate hydrochloride by adding a carbon dioxide gel to the electrode matrix as a transducer. The results showed that the sensitivity and selectivity were improved in the concentration range of MFX from 10-6 to 10-2 mol/L, and the average recovery of the developed sensor was good.

3.5 Other methods

In recent years, nuclear magnetic resonance quantification (Q-NMR) has been increasingly used in the field of pharmaceutical standards[33]. Q-NMR can be the method of choice for the quantification of innovative drugs for which no control is available. 1H-NMR was used to determine the content of meclofenoxate hydrochloride by Wang et al[18].. The method was investigated in terms of instrumental precision, method reproducibility and linearity, and the results were compared with those of the mass balance method. The study showed that the method was accurate and reliable, and provided a new reference for the quality control of the substance and the calibration of the control. WeiXing et al.[25] established a new method for the rapid determination of meclofenoxate hydrochloride in preparations using meclofenoxate hydrochloride and eosin Y in Clark-Lubs buffer (pH 3.6) to discolor the system, and meclofenoxate hydrochloride was found to comply with Biel's law in the concentration range of 2-18 μg/mL. Hu et al.[34] developed a new method for the determination of meclofenoxate based on the RRS method combined with the flow injection analysis (FIA) technique. The method was sensitive with a detection limit of 5.6 ng/mL. The method was also applied to the determination of meclofenoxate in pharmaceutical preparations, and the results were consistent with the pharmacopoeia. In addition, Zhou et al.[35] used the volumetric method to determine the content of meclofenoxate hydrochloride, and the results showed that the linearity of the determination of meclofenoxate hydrochloride was good, the mean recoveries were 99.74% and the precision was 0.50%, respectively, but compared with the high performance liquid chromatographic method, the pre-treatment steps of the volumetric method were more cumbersome, and the high performance liquid chromatographic method was more rapid and the method was more reproducible, which was generally chosen for the determination of meclofenoxate hydrochloride. The method was generally selected for the determination of meclofenoxate.

4. Conclude

In summary, most of the current detection of meclofenoxate is focused on high performance liquid chromatography and liquid chromatography-mass spectrometry, etc. Most of the testing methods for meclofenoxate are focused on the quality control of the clinical use of meclofenoxate hydrochloride for the determination of the substance and its content. The combination of multiple instruments and the integrated analysis of various coupling techniques is the mainstream direction for the detection of meclofenoxate substances, and electrochemical methods can use disposable detectors to prevent cross-contamination of the analyzed biological samples, but the stability of nanomaterials in complex media is an issue that requires attention. From the field of doping control, in addition to meclofenoxate itself, the determination of the excretion of the urinary metabolites of meclofenoxate, 4-CPA and deanol-N-oxide, can also be used to indirectly monitor meclofenoxate elimination, and the elimination kinetics of meclofenoxate's major metabolites, 4-CPA and deanol, can be further investigated in the future, with the aim of providing a certain degree of reference significance for sports drug testing and doping decision-making. In addition, in order to explore more deeply whether there are other sources of substances in the samples of meclofenamate metabolites, thus avoiding certain interference with the analysis of meclofenamate, with the continuous development of the detection technology, afterwards, there is a need to further evaluate the most suitable analytical methods for meclofenamate identification and detection, to shorten the detection time and pre-treatment procedures, which is conducive to the efficient monitoring of stimulants and to combat and control This will help to achieve efficient doping control and provide technical support to combat and control the abuse of meclofenamate.

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References

[1] Huasong Zhang, Cong Fan, Ling Li, Feiyi Liu, Shaoying Li, Linyun Ma, Yuanhao Yang, David N. Cooper, Yuedong Yang, Ronggui Hu, Huiying Zhao. Repurposing the Memory-promoting Meclofenoxate Hydrochloride as a Treatment for Parkinson's Disease through Integrative Multi-omics analysis . bioRxiv preprint - Biochemistry. 2023-04-09 , DOI: 10.1101/2023.04.07.536024

[2] Oliver Krug, Sven Guddat, Christian Görgens, Thomas Piper, Tristan Möller, Mario Thevis. Determination of urinary deanol-N-oxide excretion profiles after ingestion of nutritional supplements containing deanol. Drug Testing and Analysis，2023, 15, 1312-1318.

[3] Pieter A. Cohen, Bharathi Avula, Ikhlas Khan. The unapproved drug centrophenoxine (meclofenoxate) in cognitive enhancement dietary supplements. Clinical Toxicology, 60, 2022, 1156-1158

[4] Ni, B., Zhang, J., Zou, J., Zhao, W., & Li, J. (2010). A Simple and Sensitive HPLC Method For Quantification of the Metabolin of Meclofenoxate In Human Plasma. Journal of Chromatographic Science, 48(5), 353-357.

[5] World Anti-Doping Agency. Meclofenoxate.2006. https://www.wada-ama.org/sites/default/files/resources/files/tl01_meclofenoxate_eng_2021.pdf (03-08-2021)

[6] Guddat, S. (2006). Detection of meclofenoxate and its degradation products Dimethylaminoethanol and pchlorophenoxyacetic acid. In Recent Advances in Doping Analysis, (14), 399.

[7] Yu. A. Yugay, O. V. Grishchenko, E. A. Vasyutkina, V. P. Grigorchuk, E. N. Chukhlomina, Zh. L. Tsydeneshieva, O. D. Kudinova, Yu. L. Yaroshenko, A. I. Degtyarenko, E. P. Subbotin, V. P. Bulgakov, Yu. N. Kulchin, Yu. N. Shkryl . Influence of Growth Regulators and Different Spectra of Monochromatic Radiation on the Growth and Biosynthetic Characteristics of Callus Culture of Ipomoea batatas (L.) Lam. Russian Journal of Plant Physiology. 2024-01-26 , DOI: 10.1134/s1021443723603105

[8] Dong-X. Zhang, S. Qu, Yu-H. Liu, C. Xu, Xiao-Y. Liu, H. Kan, K. Dong, Ying-P. Wang. Application of three-dimensional material CZIF-8/CS-MS as adsorbents for the determination of plant growth regulators in Schisandra chinensis . Journal of Chromatography A. 2024-02-09 , DOI: 10.1016/j.chroma.2024.464727

[9] C. Cai, F. Yao, C. Li, Y. Xiang, P. Wu, Z. Hu, J. Wang, R. Zhu . Determination of Ten Plant Growth Regulators in Bean Sprouts by Mixed Solid Phase Extraction Columns Separation and LC-MS/MS . Processes. 2023-08-29 , DOI: 10.3390/pr11092586

[10] Ben Ouaghrem, M., de Vaugelade, S., Bourcier, S., Genty, C., Pirnay, S., & Bouchonnet, S. (2022). Characterization of photoproducts and global ecotoxicity of chlorphenesin: A preservative used in skin care products. International Journal of Cosmetic Science, 44(1), 10-19.

[11] Mario Thevis. The 41st Manfred Donike workshop on doping analysis. Drug Test Anal. 2023, 15, 1310–1311. DOI: 10.1002/dta.3601

[12] Parui, A., Biswas, S., & Roy, I. (2023). Meclofenoxate Inhibits Aggregation of Alpha-Synuclein in vitro. Protein and peptide letters.

[13] Zs-Nagy, I. (1994). A survey of the available data on a newnootropic drug. Annals of the New York Academy of Sciences, 717(1): 102-105.

[14] Lei, L. U., Xiaojiang, S. U. N., Jin, Z., Bo, X. U. E., & Hong, S. H. I. (2007). The clinical efficacy of meciofenoxate hydrochioride in treatment of patients with acute intracerebrai hemorrhage. Pharmaceutical Care and Research, 7(4), 271-273.

[15] Goergens, C., Guddat, S., Thomas, A., Wachsmuth, P., Orlovius, A.-K., Sigmund, G., Thevis, M., & Schaenzer, W. (2016). Simplifying and expanding analytical capabilities for various classes of doping agents by means of direct urine injection high performance liquid chromatography high resolution/high accuracy mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 131, 482-496.

[16] Karol Jędrejko, Oliver Catlin, Timothy Stewart, Ashley Anderson, Bożena Muszyńska, Don H Catlin. Unauthorized ingredients in “nootropic” dietary supplements: A review of the history, pharmacology, prevalence, international regulations, and potential as doping agents. Drug Testing and Analysis. 2023-06-25, DOI: 10.1002/dta.3529

[17] Nan, F., Tang, S., Sang, X., Chen, Z., Ding, H., Yan, B., Zeng, J., & Wu, L. (2021). Determination of 2-dimethylaminoethanol in human plasma after oral meclofenoxate. Chinese Journal of Pharmaceutical Analysis, 41(2), 263-268.

[18] Wang, X., Zhou, Y. & Wei, N. Y. (2019). Determination of meclofenoxate hydrochloride by nuclear magnetic resonance internal standard method. China Drug Standard, 20(02): 119-122.

[19] Feng, T., Zhang, S., Chen, Y. (2020). Progress in research on the pharmacological effects of meclofenoxate and its metabolites. Chinese Journal of New Drugs, 29(11), 1297-1301.

[20] Sun, H., Yuan, M., & Du, H. Y. (2020). Determination of chlorpheniramine in human blood by ultra fast liquid chromatography-tandem mass spectrometry. Criminal Technology, 45(01): 55-59.

[21] Rubio, A., Gorgens, C., Krug, O., Okano, M., Fedoruk, M., Ahrens, B., Geyer, H., & Thevis, M. (2021). Chromatographic-mass spectrometric analysis of the urinary metabolite profile of chlorphenesin observed after dermal application of chlorphenesin-containing sunscreen. Rapid Communicationsin Mass Spectrometry, 35(21).

[22] Zhou, Q., Li, J., & Xu, X. M. (2009). Determination of meclofenoxate hydrochloride dispersible tablets and related substances by high performance liquid chromatography. China Contemporary Medicine, 16(11): 73.

[23] Krug, O., Guddat, S., Goergens, C., Piper, T., Moeller, T., & Thevis, M. (2023). Determination of urinary deanol-N-oxide excretion profiles after ingestion of nutritional supplements containing deanol [Article; Early Access]. Drug Testing and Analysis, 15(11-12), 1312-1318.

[24] Hua, L. U., Quan-xiu, X. U., Ai-hong, S. H. I., Lin-qiu, L. I., & Bi-yang, D. (2009). Indirect Determination of Meclofenoxate Hydrochloride in Human Plasma by Capillary Electrophoresis Coupled with Electrochemiluminescence Detection. Journal of Analytical Science, 25(6), 639-642.

[25] Weixing, M. A., Yinghong, L. I. U., Ou, S. H. A., & Yan, Z. (2010). Fading Spectrophotometric Method for Determination of Meclofenoxate Hydrochloride with Eosin Y. Chinese Journal of Pharmaceuticals, 41(3), 213-215.

[26] Ting, M., Min, Z., Mao, L., & Fang, C. (2006). Determination of related substances in meclofenoxate hydrochloride for injection by high performance liquid chromatography. Journal of the Fourth Military Medical University, (20), 1919.

[27] Zhang, Y., Zhao, L., Zhao, X., Liu, Z. (2018). Determination of meclofenoxate hydrochloride and its metabolites in human plasma by high-performance liquid chromatography-tandem mass spectrometry. Biomedical Chromatography, 32(3), e4119.

[28] Wang, L., Wu, Z., Wang, T., Xie, L., & Hu, Q. (2017). Correlation study between production process and impurity profile of meclofenoxate hydrochloride for injection. Chinese Journal of Pharmaceutical Analysis, 37(7), 1304-1308.

[29] Lingyun, H. E., Hongpeng, X. I. A., Jianping, W. U., & Zeneng, C. (2007). Determination of meclofenoxate hydrochloride capsules by HPLC. Chinese Journal of Analysis Laboratory, 26(9), 74-76.

[30] Chen, G. & Huang, T. H. (2009). Content of meclofenoxate for injection and determination of related substances. Pharmacopoeia, 28(02): 243-245.

[31] Khalil, M. M., & Aleem, A. A. A. (2019). Novel Potentiometric Sensors Based on Carbon and TiO2 Nano tubes/ beta-cyclodextrin for Meclofenoxate Hydrochloride Micro Determination in Spiked Surface Water and Urine Samples. Egyptian Journal of Chemistry, 62(4), 1127-1138.

[32] Aleem, A. A. A., Khaled, E., Farghali, A. A., Abdelwahab, A., & Khalil, M. M. (2020). beta-Cyclodextrin/ Carbon Xerogel Based Potentiometric Screen Printed Sensor for Determination of Meclofenoxate Hydrochloride. International Journal of Electrochemical Science, 15(4), 3365-3381.

[33] Zhang, Q. Li, X. & Yang, H. X. (2012). Advances in the application of nuclear magnetic resonance technology in the field of pharmaceutical standards. Journal of Pharmaceutical Analysis, 32(03): 545-549.

[34] Hu, X., Xu, D., Liu, S., Liu, Z., Li, C., & Chen, P. (2010). DETERMINATION OF MECLOFENOXATE HYDROCHLORIDE BY RESONANCE RAYLEIGH SCATTERING METHOD COUPLED WITH FLOW INJECTION TECHNIQUE [Article]. Analytical Letters, 43(13), 2125-2133.

[35] Zhou, W., Li, J., & Li, Y. B. (2009). Comparison of methods for the determination of meclofenoxate hydrochloride dispersible tablets. China Pharmaceutical Journal, 6(11): 49-51.