

GLAUCOMA MEDICATION: ACETAZOLAMIDE

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Abstract

Glaucoma is an ocular disorder characterized by ocular hypertension that causes progressive and irreversible loss of vision being the second cause of blindness worldwide. Carbonic anhydrases inhibitors used as antiglaucoma agents decrease the pressure in the eyes by reducing the production of aqueous humor.

The analysed compound, acetazolamide, was obtained from acetazolamide tablets (Acetazolamide by Arena Group SA) after extraction with ethyl acetate and was characterized by appearance, colour, odour and solubility. Chemical identification was determined through Romanian Pharmacopoeia Xth edition techniques. All these tests were in accordance with the requirements of Romanian Pharmacopoeia Xth edition.

Determination of purity of acetazolamide was performed by TLC using four mixtures of solvents. We found two mixtures of solvents recommended for acetazolamide identification and purity determination: acetone-ethanol (1:1) and hexane-ethyl acetate (1:10). Partial chemical structure studies were performed by UV-VIS spectrophotometry.

Key words: glaucoma, optic nerve, acetazolamide, thin layer chromatography TLC.

INTRODUCTION

Glaucoma is a term describing a group of ocular disorders with multifactorial etiology and different pathology united by a clinically common characteristic: increased intraocular pressure. It is not a single entity and is sometimes referred to in the plural as the glaucomas. The ocular hypertension has as consequence an optic nerve circulatory disorder that causes optic atrophy and generally evolves towards progressive and irreversible loss of vision (blindness). Evidence suggests that the primary site of neurological injury is at the optic nerve head (Casson R.J. et al., 2012; Cernea P., 2001; Pop R., 2003; <http://en.wikipedia.org/Glaucoma>).

There are two main types of glaucoma: open-angle glaucoma and closed-angle glaucoma. *Open-angle glaucoma* is most common, it is painless, does not cause symptoms in the early stages and does not have acute attacks but produces progressive narrowing of the visual field and optic nerve damages with complete loss of vision in the absence of treatment. *Closed-angle glaucoma* is much more rare but gives acute crises characterized by sudden ocular pain, severe headaches, seeing halos around lights, red eye, nausea and vomiting, very high intraocular pressure (>30 mmHg) and suddenly decreased vision. Closed angle glaucoma is an emergency because vision may be lost if only 2-3 hours of onset of

symptoms but without any treatment. Glaucoma affects about 44 million people being the second cause of blindness worldwide (Beers M.H., 2003; Quigley H.A. et al., 2006; <http://www.who.int/bulletin/volumes>).

Glaucoma medications are divided into several groups based on chemical structure and pharmacologic action:

- adrenergic agonists – apraclonidine, brimonidine, dipivefrine;
- adrenergic antagonists – betaxolol, carteolol, metipranolol, levobunolol, timolol;
- parasympathomimetic agents – acetylcholine, carbachol, pilocarpine;
- carbonic anhydrase inhibitors – acetazolamide, brinzolamide, dorzolamide, methazolamide;
- prostaglandin analogs – bimatoprost, latanoprost, tafluprost, travoprost, unoproston.

The carbonic anhydrases are ubiquitous zinc enzymes present in about 14 isoforms in higher vertebrates with very different subcellular localization and tissue distribution. These enzymes catalyze the interconversion between carbon dioxide and the bicarbonate ion being involved in physiological processes such as respiration, pH and carbon dioxide homeostasis, electrolyte secretion in a variety of tissues, gluconeogenesis, lipogenesis, calcification, bone resorption. Many of these isozymes are important targets for the design of inhibitors with clinical applications (Supuran C.T. et al., 2003).

Carbonic anhydrase inhibitors suppress the activity of carbonic anhydrase and belong to two main classes of compounds: the metal complexing inorganic anions and sulphonamides. The sulphonamides are useful as antiglaucoma agents, antiepileptics, diuretics, in the management of mountain sickness, gastric and duodenal ulcers, congestive heart failure as well as diagnostic tools (Supuran C.T. et al., 2000).

MATERIAL AND METHOD

Carbonic anhydrase inhibitors used as antiglaucoma agents decrease the pressure in the eyes by reducing the production of aqueous humor. Inhibition of carbonic anhydrase in the ciliary processes of the eye decreases the aqueous humor secretion with 40-50%. Systemic administration of carbonic anhydrase inhibitors is effective but is accompanied by an increased rate of side effects: fatigue, depression, loss of appetite, weight loss, kidney stones, metallic taste, peripheral neuropathies. Therefore, there have been chemical changes that allow topical administration of these compounds (Cernea P., 2001; <http://www.webmd.com/eye-health>).

Acetazolamide (Fig. 1) sample used in this study comes from acetazolamide tablets produced by Arena Group SA (series 028408008), the

compressed substance was isolated by extraction with a suitable solvent. Because the substance is slightly soluble in the usual solvents such as acetone, ethanol, water, chloroform and ether, the ethyl acetate was chosen as solvent for extraction. In this regard, 20 tablets of acetazolamide (250 mg acetazolamide/tablet) after shredding to a fine powder were extracted one time with ethyl acetate (partial extraction, 50 ml of ethyl acetate). The organic layer was filtered and then evaporated to dryness under reduced pressure to give approximately 2.5 g of pure substance on which the following tests were performed: organoleptic study, solubility, chemical identification, thin layer chromatography, ultraviolet-visible spectral study (Mance R., 2010).

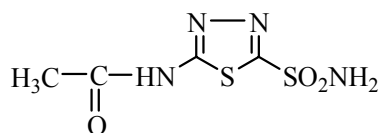


Fig. 1. Acetazolamide
N-[5-sulfamoyl-(1,3,4-thiadiazol-2-yl)]-acetamide

RESULTS AND DISCUSSION

According to Romanian Pharmacopoeia Xth edition (RPX), acetazolamide is a crystalline powder, white or yellow-white, odorless and tasteless (source: Romanian Pharmacopoeia Xth edition, 1993).

Organoleptic study was determined in accordance with working techniques provided by Romanian Pharmacopoeia Xth edition. The results obtained are shown in Table 1.

Table 1

Organoleptic characteristics of acetazolamide

COMPOUND	APPEARANCE	COLOUR	ODOUR
Acetazolamide (RPX)	crystalline powder	white or yellow-white	odorless
Acetazolamide (Arena)	crystalline powder	white	odorless

Solubility in different solvents was determined in accordance with Romanian Pharmacopoeia Xth edition. The solubility of acetazolamide is presented in Table 2.

Chemical identification was determined in accordance with Romanian Pharmacopoeia Xth edition (decomposition of 1,3,4-thiadiazole ring with

identification of H₂S produced and acetazolamide-CuSO₄ complex formation) and was in agreement with RPX techniques.

Table 2

SOLVENTS	ACETAZOLAMIDE
acetone	slightly soluble
ethanol	sparingly soluble
water	very sparingly soluble
dimethylformamide	very soluble

Thin layer chromatography (TLC) used solutions of 10⁻⁴-10⁻⁵ M of acetazolamide in ethyl acetate. The following mixtures of solvents were used (Table 3).

Table 3

MIXTURES OF SOLVENTS	COMPONENTS	RATE
S ₁	acetone-ethanol	1:1
S ₂	ethyl acetate-ethanol	1:1
S ₃	hexane-ethyl acetate	1:10
S ₄	ethyl acetate-ethanol	2:1

Merck chromatographic plates with dimensions of 20x20 cm and silica gel 60 F254 applied on polyester were used. The samples were spotted at a distance of 1 cm from the edge of TLC plates using microcapillary. Migration was performed in a tightly closed flask saturated by the vapors of solvents. The development over a distance of 12 cm led to the separation of a single area.

Table 4

MIXTURES OF SOLVENTS	R _f
S ₁	0.53
S ₂	0.81
S ₃	0.60
S ₄	0.84

Identification of acetazolamide in the test sample was based on R_f values after highlighting the spots in UV light at λ = 254 nm (Table 4). Ultraviolet light has been selected as visualizing agent because the 1,3,4-

thiadiazole ring system shows, due to chromophore C=N-N=C, an intense absorption in UV. In the UV light, the substance fluoresces blue-violet.

The purity condition of a compound studied by TLC requires the appearance of a single spot on the chromatographic plate. No additional spots were visualized on TLC.

Among the developing mixtures, S₁ and S₃ are most recommended for the acetazolamide study with R_f values between 0.53-0.60, the spots being well separated and with oval-rounded shapes.

Ultraviolet-visible spectral study of acetazolamide was performed with a Beckman DU-64 spectrometer using a solution 0.001% m/v of acetazolamide in 0.1M HCl. The spectrum was recorded for 200-400 nm area and has a maximum absorption band in the 220-300 nm with a maximum at 265 nm and absorbance A = 2.92. Molecular extinction coefficient intensity (ϵ) for a determined absorption is proportional to the probability of electronic transition. Taking into account the structure of acetazolamide, the absorption band observed in the UV-VIS spectrum is corresponding to n \rightarrow π^* and $\pi\rightarrow\pi^*$ electronic transition.

CONCLUSIONS

The analysed compound, acetazolamide, was obtained from acetazolamide tablets produced by Arena Group SA after extraction with ethyl acetate and was characterized by appearance, colour, odour and solubility. Chemical identification was determined through Romanian Pharmacopoeia Xth edition techniques. All these tests were in accordance with the requirements of Romanian Pharmacopoeia Xth edition.

Determination of purity of acetazolamide was performed by TLC using four mixtures of solvents. In UV light at 254 nm, on the TLC plates is only one coloured area showing blue-violet fluorescence and corresponding to pure acetazolamide. TLC test serves not only to determine the purity of the substance but also to identify it through R_f values. The most recommended mixtures of solvents for acetazolamide identification and purity determination are acetone-ethanol (1:1) and hexane-ethyl acetate (1:10) with R_f values between 0.53-0.60.

Acetazolamide structure was studied using UV-VIS spectrum recorded for 200-400 nm area with a maximum absorption at 265 nm in agreement with the requirements of Romanian Pharmacopoeia Xth edition.

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