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Trends in development and quality assessment of pharmaceutical formulations - F2α analogues in the glaucoma treatment

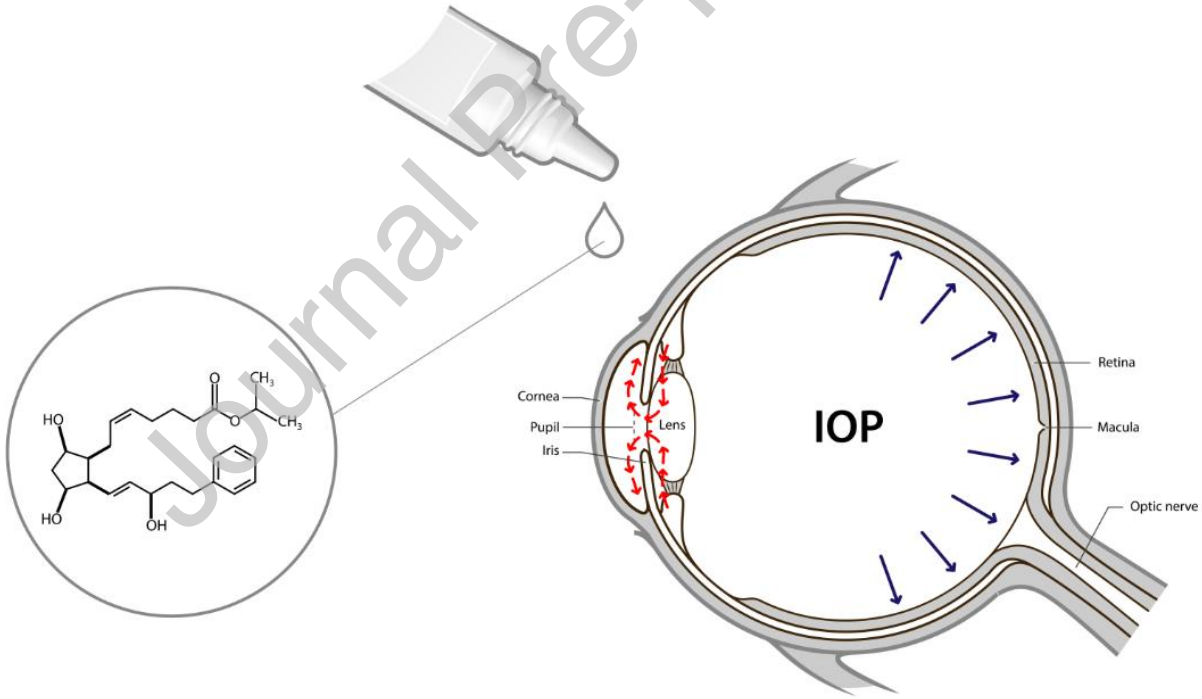
Katarzyna Asendrych-Wicik[1, 2]*, Jakub Zarczuk[3, 4]*, Katarzyna Walaszek[5], Tomasz Ciach[4], Magdalena Markowicz-Piasecka[2]

[1] Analytical Laboratory, Research and Development Department, Polfa Warszawa S.A., ul. Karolkowa 22/24, 01-207 Warsaw, Poland
[2] Department of Pharmaceutical Chemistry, Drug Analysis and Radiopharmacy, Medical University of Łódź, ul. Muszyńskiego 1, 90-151 Łódź, Poland
[3] Liquid Dosage Form Laboratory, Research and Development Department, Polfa Warszawa S.A., Karolkowa 22/24, 01-207, Warsaw, Poland
[4] BioMedical Engineering Laboratory Department of Biotechnology and Bioprocess Engineering, Faculty of Chemical and Process Engineering, Warsaw University of Technology, ul. Waryńskiego 1, 00-645, Warsaw, Poland
[5] Technical Research and Development Quality Assurance, Polpharma Bioologics., ul. Spółdzielcza 4, 05-850 Duchnice, Poland

* These authors contributed equally to this work and are co-first authors

e-mails: k.asendrych-wicik@polfawarszawa.pl, j.zarczuk@polfawarszawa.pl, katarzyna.alicja.walaszek@gmail.com, tomasz.ciach@pw.edu.pl, magdalena.markowicz@umed.lodz.pl

Graphical abstract



Abstract:

The ocular delivery route presents a number of challenges in terms of drug administration and bioavailability. The low bioavailability following topical ophthalmic administration shows that there is a clear need for in-depth research aimed at finding both more efficacious molecules and formulations precisely targeted at the site of action. Continuous technological development will eventually result in improved bioavailability, lower dosages, reduced toxicity, fewer adverse effects, and thus better patient compliance and treatment efficacy. Technological development, as well as increasingly stringent quality requirements, help stimulate analytical progress. This is also clearly evident in the case of medicinal

products used in the treatment of glaucoma, which are the subject of this review. Impurity profiling of PGF2α analogues, either in the pure substance or in the finished formulation, is a crucial step in assessing their quality. The development of specific, accurate and precise stability-indicating analytical methods for determining the content and related substances seems to be an important issue in relation to this tasks. A total of 27 official and in-house analytical methods are presented that are used for the analysis of latanoprost, travoprost and bimatoprost. The conditions for chromatographic separation with UV or MS/MS detection and the available results obtained during method validation are described. In addition, several aspects are discussed, with particular emphasis on the instability of the analogues in aqueous solution and the phenomenon of isomerism, which affects a potentially large number of degradation products.

Key words: glaucoma, prostaglandin analogue, drug formulation, analytical method

1. INTRODUCTION

After cardiovascular and respiratory diseases, diseases of the eye are among the most prevalent in the general population. Based on recent reports and meta-analyses, the incidence of glaucoma is rising. As a disease, glaucoma is one of the most persistent causes of vision impairment or blindness among disorders of the vision organ (Quigley and Broman, 2006; Tham et al., 2014). Ageing of the worldwide population is also a significant factor in the occurrence of glaucoma (Bourne et al., 2010), which leads to irreversible damage of the optic nerve cells and, ultimately, blindness. Among the most commonly reported signs is the loss of peripheral vision; severe pain is uncommon (Hu et al., 2014). This disease can be caused, e.g. by a build-up of pressure in the eye when fluid is unable to drain properly (Kass et al., 1980), and this is the leading risk factor in glaucoma development (Kara et al., 2017).

Once diagnosed, there are three main approaches to the treatment of glaucoma: through the use of topical drugs such as eye drops, laser surgery (trabeculoplasty) to facilitate ocular fluid drainage, and filtering surgery (also called incisional surgery) (Ph Denis et al., 2007; Gazzard et al., 2019; Weinreb and Khaw, 2004). There are also newly approved therapies, such as control release systems, e.g. biodegradable implants with bimatoprost (Craven et al., 2020) or latanoprost (Giarmoukakis et al., 2013). Although laser therapy is still the method of choice (Gazzard et al., 2001), of the glaucoma therapies available on the market, topical drugs such as eye drops are the most commonly used. These include a few groups of active substances, each with a different mechanism of action. Prostaglandin analogues, e.g. latanoprost, travoprost, tafluprost, unoprostone, and bimatoprost, are currently the front-line medication for the treatment of glaucoma. They exert an effect on metalloproteinases and influence the shape of the connective cells as a result of the increase in aqueous outflow and decreases in intraocular pressure (Toris et al., 2008). The second group of anti-glaucoma drugs contains β-blockers, with timolol being the most widely used β-blocker for Intraocular pressure (IOP) reduction. The exact mechanism of action of timolol is not known, but it is believed to suppress aqueous production (Bromberg et al., 1980; Kiland et al., 2004).

Another group consists of carbonic anhydrase inhibitors such as, for example, substances based on the sulfonamide structure. Acetazolamide, the most frequently used anhydrase inhibitor, was developed as a weak diuretic drug. Inhibition of carbonic anhydrase leads to sodium and hydrogen ion disorder and increased water excretion through the kidneys. In the early 1990s, dorzolamide and brinzolamide were developed as more specific drugs. They act by reducing aqueous production by ciliary cells (Pfeiffer, 1997) after topical administration of the drug solution.

Alpha adrenergic drugs, such as brimonidine (an α2 adrenergic receptor agonist), are also used in the treatment of glaucoma and are characterized by weaker systemic adverse reactions (Rahman et al., 2010). The brimonidine-induced reduction in IOP is associated with a decrease in aqueous flow and an increase in uveoscleral outflow (Toris et al., 1999). Among the groups of IOP-lowering drugs listed above there are also the newly developed Rho kinase inhibitors (serine/threonine-protein kinase), e.g.

netarsudil, ripasudil (Wang and Chang, 2014) and latanoprostene bunod, which offers the synergistic combination of latanoprost and nitric oxide (Hoy, 2018).

There are also other drug combinations, such as a beta blocker with a prostaglandin or a carbonic anhydrase inhibitor with a beta-blocker as an adjunct (Li et al., 2016). Combining IOP–lowering drugs offers the possibility of fewer daily administrations, leading eventually to better patient compliance (e.g. a dorzolamide formulation administered three times daily vs. dorzolamide + timolol administered twice daily).

The aim of our paper is to present the current state of knowledge and new trends in the development of pharmaceutical formulations containing prostaglandin $F_{2\alpha}$ analogues used in the treatment of glaucoma, taking into account analytical aspects that will ensure a high degree of quality in the products discussed. In particular, we show how the continuous progress that is being achieved in improving pharmaceutical formulations will enable products to be obtained that are characterized by improving bioavailability with an attendant reduction in adverse effects. Moreover, we shed light on some of the difficulties and limitations that are being encountered in the development and quality control of pharmaceutical products that result from the physicochemical properties of this group of drugs.

2. BASIC CHARACTERISTIC OF PROSTAGLANDINS

Eicosanoids are the products of the multistep arachidonic acid transformation pathway. They are potent autocoids that act locally and are synthesized *de novo* from lipid precursors. The class of eicosanoid includes prostaglandins, thromboxanes, leukotrienes and lipoxins. As regards their chemical structure, they are fatty acid derivatives with a molecular structure based on 20 carbon atoms that share a prostanoic acid skeleton (Di Costanzo et al., 2019). Prostaglandins are not stored in tissues in a preformed state but are generated from membrane-released arachidonic acid (AA) and released in response to a given stimulus, such as cytokine, growth factors, and other pro-inflammatory stimuli (Seo and Oh, 2017a).

Prostaglandins are mainly produced by metabolism with the aid of the enzyme cyclooxygenase (COX). The biological activity of prostaglandins is directly related to the mechanism of their formation, as cyclooxygenase-1 (COX-1) is present in most tissues as a constitutive form, involving the formation of prostanoids in order to maintain homeostasis in the human body (e.g. by modulating vascular responses), while the action of cyclooxygenase 2 (COX-2) is strongly induced by inflammation and leads to the formation of pro-inflammatory prostaglandins. For this reason, compounds with COX-2 inhibitory properties have found application in medicine and are represented by the group of non-steroidal anti-inflammatory drugs (NSAIDs). The principal prostaglandin, PGH_2, is produced by both COX isoforms and is the common substrate of several specific isomerases and synthases that produce PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and TXA_2 (E. Ricciotti, 2011).

The prostaglandins are divided according to the chemical groups on the pentane ring into types designated by the letters E, F, A, B, C and D (Piper, 1977).

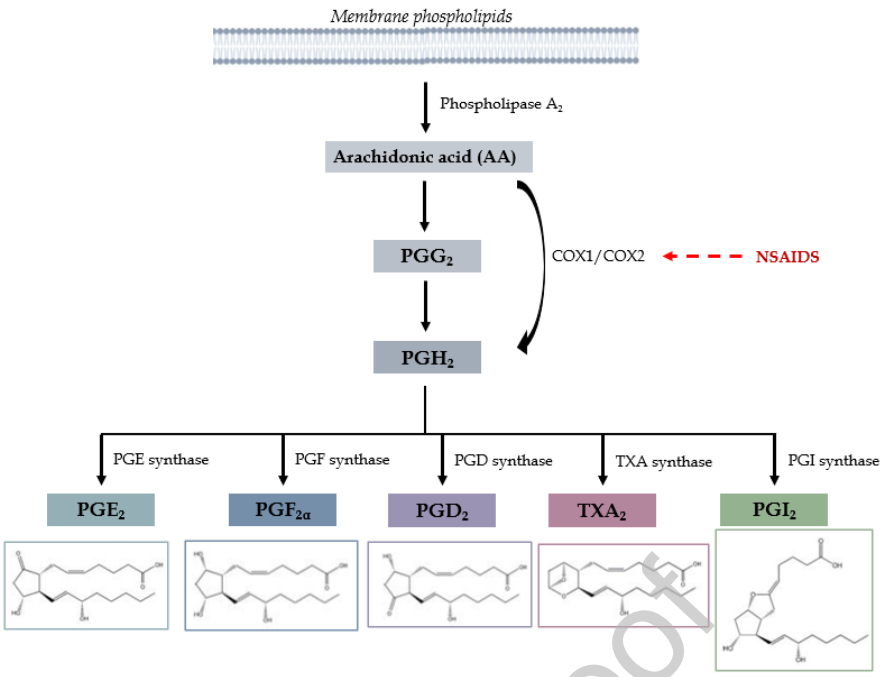


Figure 1. *Synthesis of prostanoids from arachidonic acid (AA).*

As shown in Figure 1, E-type prostaglandins have a ketone group at C9 and a hydroxyl group at C11, while in D-type prostaglandins these groups are reversed. F-type prostaglandins have hydroxyl groups at C9 and C11 on the pentane ring and form two isomers designated α and β (Piper, 1977). In addition to the differences in substituent in the pentane ring, prostaglandins differ also in the number of unsaturated double bonds. The mono-unsaturated prostaglandins have only one double bond at C13-C14, the bis-unsaturated have a C5-C6 double bond in addition, and the tris-unsaturated also have a double bond at C17-C18 (Piper, 1977).

Prostanoids, including prostaglandins, are considered to exert local effects via specific receptors located on the cell surface. However, the specificity of these receptors should be borne in mind, and also the accompanying significant overlap that contributes to the various or even opposite responses of tissues to PGs (McCracken, 2005). Prostaglandins exert their biological effects via rhodopsin-like seven transmembrane-spanning G-protein-coupled receptors (GPCRs). The activation of these receptors contributes to the significant changes in several intracellular pathways that mediate the effects in individual cells (E. Ricciotti, 2011). The types of prostaglandin receptors, their subtypes, second messengers and main physiological functions are summarized in Table 1.

Individual types of prostaglandins have different biological functions and differ in the number and position of their double bonds. Despite their pro-inflammatory potential, under the physiological conditions of homoeostasis, the wide range of physiological functions performed by prostaglandins is the basis for considering these compounds in the context of the treatment of a variety of diseases. So far, prostaglandins and their analogues have found application: (i) in ophthalmology, as compounds reducing intraocular pressure; (ii) in gynaecology, as compounds enhancing uterine contractions; (iii) in neonatology, as compounds supporting the patency of the arterial tract in newborns; (iv) in the treatment of diseases of the gastrointestinal tract, as compounds reducing the risk of ulcer formation; and (v) in peripheral vascular disease, as compounds improving the blood flow parameters and the condition of the blood vessels (Chung-Davidson et al., 2013; Lee et al., 2020; McCracken, 2005; Seo and Oh, 2017b).

Table 1. *Function and signal transduction of prostanoids through their respective receptors* (Chung-Davidson et al., 2013; Lee et al., 2020; McCracken, 2005; Seo and Oh, 2017b).

Type of prostanoid	Type of receptor	Subtype of receptors	G-protein coupled	Second messenger	Main function
TXA_2	TXA	TP_α, TP_β	Gq, G13, Gh, Gs(TPα), Gi(TPβ)	$IP_3/DAG/Ca^{2+}$, RhoGEF	Involved in vasoconstriction and platelet aggregation; modulates endothelial cell responses.
PGD_2	PGD	DP_1, DP_2 ($CRTH_2$)	Gs Gi	↑ cAMP ↓cAMP, ↑Ca^{2+}	Involved in the regulation of the CNS, non-rapid eye movement sleep, chemotaxis, allergy-induced asthma.
PGE_2	PGE	EP1, EP2, EP3, EP4	Gq Gs Gi, G12 Gs	↑$IP_3/DAG/Ca^{2+}$ ↑ cAMP ↓cAMP, ↑Ca^{2+} ↑ cAMP	Mediates cellular processes in pro-inflammatory and anti-inflammatory directions; involved in pain response, ovulation, fertilisation, fever, bone resorption, and stimulation of neurogenesis.
PGI_2	PGI	IP_1, IP_2	Gs Gs	↑ cAMP ↑ cAMP	Acts as a potent vasodilator and an inhibitor of platelet aggregation. Involved in leukocyte adhesion, proliferation of vascular smooth muscle cells, and regulation of cardiovascular homeostasis.
$PGF_{2\alpha}$	PGF	FP_A, FP_B	Gq	↑$IP_3/DAG/Ca^{2+}$	Involved in oogenesis, ovulation, luteolysis, contraction of uterine smooth muscle, and initiation of parturition. It also functions as an abortifacient.

Abbreviations: IP_3 - inositol trisphosphate; DAG - diacylglycerol; cAMP - cyclic adenosine monophosphate; CRTH2 - chemoattractant receptor-homologous molecule expressed on receptor T(H)2 cells; CNS - central nervous system. An upward arrow (↑) indicates an increased intracellular signalling pathway, and a downward arrow (↓) denotes a decrease in an intracellular signalling pathway.

3. PHARMACEUTICAL APPLICATIONS OF PROSTAGLANDIN ANALOGUES

3.1. PGAs in ophthalmology

The fact that a number of PGs are synthesised in the iris and in the ciliary body has been known since the 1970s. PGE_2 and PGF_α are present mainly in the eye and are released following any eye trauma. The physiological functions of PGs in the eye include reduction of intraocular pressure (IOP), induction of vasodilatation, elevation of vascular permeability, and pupil constriction (Dams et al., 2013b). The hypotensive activity of PGs has been shown to stem from an enhanced uveoscleral outflow, with minor effects on trabecular outflow and aqueous flow (Carol B.Toris, B'Ann T.Gabelt, 2008). The authors suggest that this enhanced outflow is caused by the effects on the matrix metalloproteinases and remodelling of the extracellular matrix. This in turn affects the permeability of tissues associated with the outflow pathways, resulting in changes in outflow resistance and outflow rate (Carol B.Toris, B'Ann T.Gabelt, 2008).

Discovery of the effect that PGs have on IOP contributed to the design and synthesis of PG analogues (PGAs) that were found to possess extraordinary efficacy and relatively acceptable side effects (Dams et al., 2013b; Morrone et al., 2015). Ophthalmology is a field in which PGAs play a significant role, due not only to the multitude of compounds used, but also to the very large number and wide availability of preparations and/or formulations. Of all the prostaglandins and their analogues available on the market,

four are used in ophthalmology, including latanoprost, travoprost, bimatoprost and tafluprost. These agents are used as first-line drugs in the treatment of ocular hypertension (OH) and glaucoma (Dams et al., 2013b). The first commercially available PGA for glaucoma treatment was unoprostone isopropyl in Japan in 1994. However, this drug is no longer used owing to its limited efficacy and twice-daily administration regime (Dams et al., 2013b).

The available results of meta-analyses of their efficacy tend to bear out that PGAs are all fairly equal in terms of their hypotensive activity, though some do suggest that certain preparations have a slight, if clinically insignificant, superiority over the others (Dams et al., 2013b). However, one of the latest studies reveals that a newly-marketed PGA, tafluprost, can substantially reduce IOP (16.2 mm Hg at baseline down to 14.8 mm Hg) in subjects previously treated with other PGAs – latanoprost, travoprost or bimatoprost – as monotherapy (Kimmich and Hommer, 2011). Also, animal studies conducted by Kurashima *et al*. (Kurashima H, Kurashima H, 2012) have confirmed that switching therapy from latanoprost to tafluprost reduced IOP significantly, and that tafluprost's positive effect disappeared on switching back to latanoprost. The authors concluded that tafluprost may offer non-responders a powerful alternative to latanoprost (Kurashima H, Kurashima H, 2012).

Taking into account the indications, all the analogues are used in the form of eye drops, and the individual compounds differ in their duration of action and the mechanism by which they reduce intraocular pressure. Particular preparations of PGAs may also differ in the type of packaging used for drop dispensing, which directly affects their storage properties and the type of preservatives that need to be used, which may result in interaction with other preparations.

Latanoprost, an ester prodrug analogue of $PGF_{2\alpha}$ with high affinity for FP subtype receptors, was, in 1996, the second PGA to come onto the market. Latanoprost is more lipophilic than its parent prostaglandin and therefore offers better corneal penetration. The drug is completely hydrolysed after absorption by the cornea, and there are no other metabolic pathways for the drug in the eye. In the systemic circulation, the drug's peak concentration occurs after 2 hours, and metabolism of the drug includes a β-oxidation reaction in the liver. Latanoprost lowers IOP by increasing the uveoscleral outflow, with only a slight alteration, if any, in the conventional (trabeculo-canalicular) aqueous outflow and no effects on the retinal vasculature or on blood-aqueous barrier permeability (Patel and Spencer, 1996; Russo Andrea, Riva Ivano, Pizzolante Teodoro, Noto Federico, Quaranta, 2008). The latanoprost-induced reduction in IOP is dose-dependent, and after a single dose of latanoprost 0.005% these effects last for up to 20 to 24 hours, which means that the drug can be administered once daily (Patel and Spencer, 1996).

Travoprost, like latanoprost, is an ester prodrug of $PGF_{2\alpha}$ that is hydrolysed by corneal esterases into its active free-acid form. Once hydrolysed in the eye, travoprost acid interacts with FP receptors in the ciliary muscle and the trabecular meshwork (Philippe Denis et al., 2007). *In vitro* studies have shown that travoprost exhibits greater affinity and selectivity for FP receptors than for other PG receptors, and its binding affinity is higher than that of other PGAs, like latanoprost or bimatoprost (Philippe Denis et al., 2007). FP receptor-mediated intracellular signals contribute to increased production of some matrix metalloproteinases (MMPs), which are MMP-1, -2, -3 and -9, in cultured human ciliary smooth muscle cells. The changes in the extracellular matrix of the ciliary body, and subsequent newly-formed and increased spaces between the ciliary muscle fibre bundles are responsible for the IOP-lowering effects (Philippe Denis et al., 2007). Travoprost is an effective IOP-lowering medication. Used at a concentration of 0.004%, the drug provides a 6.5-9.0 mm Hg reduction in the IOP (Wadhwani et al., 2016). Clinical studies have shown that the efficacy of this agent is equivalent to that of other PGAs, and is at least as effective as combination preparations pairing timolol with latanoprost or dorzolamide (Philippe Denis et al., 2007).

Bimatoprost differs from other PGAs in that it has a slightly different chemical structure. Latanoprost and travoprost both have an isopropyl ester group at the α-chain carbon atom C-1, and they are regarded as prodrugs because they are hydrolysed by esterases to the corresponding free acids. Bimatoprost, on

the other hand, has an ethyl amide group at the α-chain carbon atom C-1. For this reason bimatoprost is classed as a prostamide analogue (Dams et al., 2013b). Although bimatoprost is related to $PGF_{2\alpha}$it does not act through any known prostaglandin receptors. The drug selectively mimics the effects of the newly-discovered biosynthesised substances, prostamides (Craven and Alzuhairy, 2014). Animal studies have shown that both a prostamide and prostanoids receptor may be activated by bimatoprost (Shafiee et al., 2013). Some recent studies reviewed by Craven and Alzuhairy (Craven and Alzuhairy, 2014) report that when bimatoprost is injected into the anterior chamber of the eye it lowers IOP significantly, suggesting the involvement of prostamide receptor in the biological activity of this agent.

Tafluprost is the last of the PG analogues marketed for the treatment of open-angle glaucoma, in addition to ocular hypertension. Importantly, tafluprost 0.0015% is the first topical prostaglandin approved by the Food and Drug Administration that does not contain the widely used preservative, benzalkonium chloride (BAK) (Swymer and Neville, 2012). The mechanism of pharmacological activity of this agent is analogous to the that of the drugs mentioned earlier, latanoprost and travoprost. Tafluprost is an ester prodrug that is hydrolysed in the eye by esterases into the active metabolite, tafluprost acid (Takagi et al., 2004). The acid compound is further metabolised by β-oxidation and phase II conjugation. The drug is quickly eliminated from the body (levels are undetectable in 30 minutes) (Swymer and Neville, 2012). In comparison studies, no significant differences are reported between latanoprost, travoprost and bimatoprost (Eisenberg et al., 2002; Parrish et al., 2003). Differences in side effects such as conjunctival hyperaemia and eyelash growth are reported to favour latanoprost, which has fewer adverse reactions compared to bimatoprost or travoprost (Beckers et al., 2008; Eisenberg et al., 2002).

The potential side effects of PGAs are summarised in Table 2. Local side effects are common with all PGAs, and they have therefore been listed separately. The currently available literature also shows that some of the side effects of PGAs are used in the treatment of certain diseases. For example, $PG_{F2\alpha}$ has been studied for its use in the treatment of alopecia and hypopigmentary disorders (Choi et al., 2015).

Table 2. *Side effects of PG analogues.*

PG analogue	Local side effects	Systemic side effects	Ref.
Latanoprost	Cosmetic problems: superficial conjunctival, episcleral blood vessel hyperaemia; dry eye; darkening of eyelids skin; darkening and elongation of eyelashes, discomfort, photosensitivity, excessive lacrimation. These side effects are equally characteristic of all PGAs. Side effects related to sight: conjunctival hyperaemia, iridial cysts (↑L) and ciliary body cysts; eyelid hyperpigmentation; anterior uveitis; cystoid macular oedema; reactivation of herpes simplex keratitis (↑B, ↑L); decrease in central corneal thickness (↑B, ↑L), periocular pigmentation; iris hyperpigmentation (↑L); conjunctival hyperaemia (↑T, ↑B); uveitis, inflammatory reactions;	Minimal systemic adverse effects; respiratory complications, mild headache, nausea, vomiting, negligible effects on mean heart rate and blood pressure	(Patel and Spencer, 1996; Perry et al., 2003)
Travoprost		Well-tolerated systemically; incidental nausea and vomiting; comparable safety to latanoprost; no effects on the cardiovascular or pulmonary systems, no changes in haematology, blood chemistry, or urinalysis laboratory values	(Philippe Denis et al., 2007)
Bimatoprost		Headache; hypersensitivity reaction, hypertension, abnormal liver tests, hypertrichosis	(Craven and Alzuhairy, 2014; Wirta et al., 2011)
Tafluprost		No changes in laboratory values, heart rate, blood pressure, vital signs, or electrocardiographic parameters; drug is well tolerated	(Dams et al., 2013b; Pozarowska, 2010)

↑L – higher frequency of prevalence with latanoprost administration; ↑T – higher frequency of prevalence with travoprost administration; ↑B – higher frequency of prevalence with bimatoprost administration.

3.2. Other medical applications of PGAs

Prostaglandin analogues are also used in other fields of medicine. For instance, misoprostol is a synthetic analogue of prostaglandin E_1 (PGE_1), which under physiological conditions protects the gastric mucosa by inhibiting the secretion of gastric juice and hydrochloric acid, while at the same time increasing the secretion of mucus and bicarbonate. For this reason, the main indication for the use of misoprostol is the prophylaxis of gastric and duodenal ulceration caused by the use of non-steroidal anti-inflammatory drugs (NSAIDs). Apart from its application in the treatment of gastric problems, misoprostol can also be used off-label for a variety of indications in obstetrics and gynaecology, i.e. medication abortion, medical management of miscarriage, induction of labour, cervical ripening prior to surgical procedures, and the treatment of postpartum haemorrhage (Allen and Brien, 2009).

The possibility of using prostaglandins in gynaecology results also from the action of prostaglandin E_2, which is a powerful stimulant of uterine contractions. Dinoprostone, a synthetic analogue of prostaglandin E_2, comes in the form of a cervical gel and is used in preparation for inducing labour at the requisite time for obstetric or internal reasons. This drug initiates and increases the strength and rhythm of uterine contractions and helps to relax, smooth and widen the cervix (Bakker et al., 2017).

Alprostadil is a prostaglandin E_1 analogue with a multidirectional action including vasodilation, especially of the arterial ducts of newborns, which is directly used in treatment. In addition, it inhibits the secretion of gastric juice, reduces platelet aggregation and stimulates the contractile activity of the intestinal and uterine smooth muscle. PGE_1 also binds to receptors in the corpus cavernosum, increases the concentration of cyclic AMP and, as a result, leads to the relaxation of the smooth muscle, increased blood flow and the formation of an erection. For this reason, alprostadil can be used as a topical preparation in the treatment of erectile dysfunction. Alprostadil is also used for maintenance treatment in newborns requiring cardiac surgery. Administration of alprostadil is intended to keep the ductus arteriosus open and to prevent its premature closure until necessary surgery (Anaissie and Hellstrom, 2016). In turn, alprostadil as a complex with alfadex (cyclodextrin α) is used in the treatment of peripheral vascular diseases, including chronic peripheral arterial occlusive disease (PAOD) or limb ischemia, although its efficacy is questionable (Lawall et al., 2017).

Current research is also focused on the development of novel PG analogues (Bhoot, 2020). For instance, latanoprostene bunod is a nitric oxide (NO)-donating prostaglandin $F_{2\alpha}$ analogue approved in the USA in 2017 as an IOP-lowering agent in subjects with open-angle glaucoma (OAG) or ocular hypertension (Hoy, 2018). Furthermore, the long-term safety and intraocular pressure-lowering efficacy of DE-117 ophthalmic solution as monotherapy has been evaluated in clinical trials. This compound is a PGE_2 receptor agonist, improves uveoscleral outflow, and lowers collagen deposition in trabecular meshwork TM cells (Bhoot, 2020).

4. ASPECTS OF PROSTAGLANDIN ANALOGUE FORMULATION

4.1. Active substance solubility vs. drug product formulation

Prostaglandin analogues used in the treatment of glaucoma belong to the group of substances that are practically insoluble in water. They have lipophilic properties (based on log*P*) that give them the capability to effectively penetrate cell barriers (corneal epithelium) (Sekine et al., 2018).

Table 3. *Comparison of prostaglandin analogue LogP values.*

Active ingredient	Log*P* value	Log*P* value (Reaxys; Lipinski's rules component)
Latanoprost	4.3 (Sekine et al., 2018)	4.40
Travoprost	4.02 (Stratton et al., 2015)	4.57
Tafluprost	n/a	4.51
Unoprostone isopropyl	5.19 (Stratton et al., 2015)	5.39
Bimatoprost	3.4 (Stratton et al., 2015)	3.15

According to the Biopharmaceutical Classification System (BCS), they belong to the second group, are practically insoluble in water and show high permeability (Figure 2).

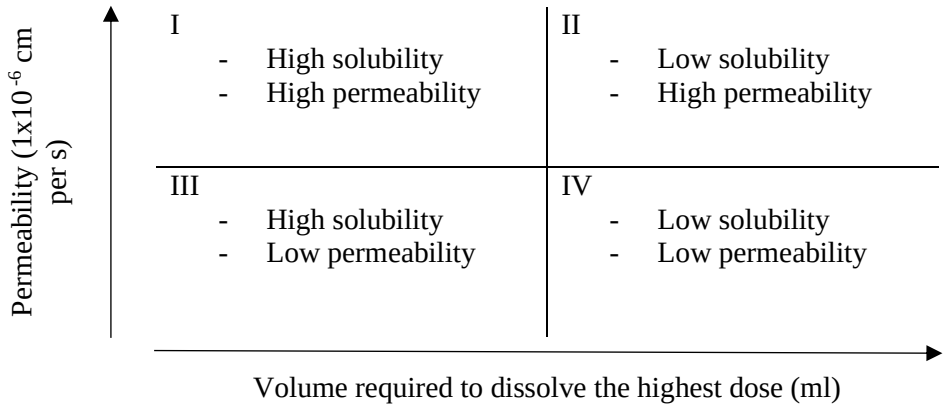


Figure 2. *Biopharmaceutical Classification System (BCS); characterisation of drugs based on solubility and permeability.*

In the pharmaceutical dosage form of eye drops the prostaglandin analogues are in the form of a pro-drug. Latanoprost, travoprost and tafluprost are inactive isopropyl esters of, respectively, latanoprost, travoprost and tafluprost acid, while bimatoprost is an ethyl amide. Their lipophilic nature allows them to effectively permeate via the cornea, where they are hydrolysed to the acid form and "transformed" into the biologically active compounds (Hejkal and Camras, 1999). They act as prostanoid receptor agonists. A variety of precorneal conditions influence active substance availability on delivery to the eye surface, e.g. drug formulation drainage, tear production and turnover, blinking, absorption into the systemic circulation by conjunctival blood vessels as well as the lymphatic circulation and, finally, efflux pumps (Hariharan et al., 2009; Ramsay et al., 2018). Thus, stabilisation of prostaglandin analogues in the lipophilic state in formulations neutral to the eye play an important role in the successful delivery of these drugs. In fact, it involves technological challenges as regards the dosage form, given that prostaglandin analogues are virtually insoluble in water. Various additives and techniques are used to introduce them into the aqueous formulations. The most important ingredients of the prostaglandin formulations are solubilisers – most commonly surfactants (Jiao, 2008). Examples of substances that increase prostaglandin solubility in water are shown in Tables 4-7.

Table 4. *Medicinal product composition – Xalatan, eye drops; 50 µg/ml solution.*

	Substance	[mg/ml]	Function
1	Latanoprost	0.05	Active substance
2	Benzalkonium chloride	0.2	Preservative/Solubiliser
3	Sodium chloride	-	Tonicity agent
4	Sodium dihydrogen phosphate monohydrate	-	Buffer
5	Disodium phosphate	-	Buffer
6	Hydrochloric acid	q.s.	To adjust pH
7	Sodium hydroxide	q.s.	To adjust pH
8	Water for injection	to 1 ml	Solvent

Table 5. *Medicinal product composition – Travatan, eye drops; 40 µg/ml solution.*

	Substance	[mg/ml]	Function
1	Travoprost	0.04	Active substance
2	Propylene glycol	7.5	Solubiliser
3	Polyoxyethylene hydrogenated castor oil 40 (HCO-40)	2.0	Solubiliser
4	Polyquaternium-1	0.01	Preservative
5	Boric acid	-	Buffer
6	Mannitol	-	Tonicity agent
7	Sodium chloride	-	Tonicity agent
8	Hydrochloric acid	q.s.	To adjust pH
9	Sodium hydroxide	q.s.	To adjust pH
10	Purified water	to 1 ml	Solvent

Table 6. *Medicinal product composition – Taflotan, eye drops; 15 µg/ml* solution.

	Substance	[mg/ml]	Function
1	Tafluprost	0.015	Active substance
2	Glycerol	-	Tonicity agent
3	Sodium dihydrogen phosphate dihydrate	-	Buffer
4	Disodium edetate	-	Chelating agent
5	Polysorbate 80	-	Solubiliser
6	Hydrochloric acid	q.s.	To adjust pH
7	Sodium hydroxide	q.s.	To adjust pH
8	Water for injection	to 1 ml	Solvent

Table 7. *Medicinal product composition – Vyzulta eye drops; 0.024%* solution.

	Substance	[mg/ml]	Function
1	Latanoprostene bunod	0.0024	Active substance
2	Benzalkonium chloride	0.2	Preservative/Solubiliser
3	Polysorbate 80	-	Solubiliser
4	Glycerol	-	Tonicity agent
5	Disodium edetate	-	Chelating agent
6	Citric acid	-	Buffer
7	Sodium citrate	-	Buffer
8	Purified water	to 1 ml	Solvent

However, from a technological perspective, poorly soluble drugs present more opportunities where drug formulation is concerned than obstacles. Various options are available as regards the dosage form (e.g. solution, emulsion, suspension) and additives e.g. solubilisers, polymers such as viscosity enhancers, can be introduced in order to obtain an effective and stable dosage form. The authors of Patent US008772337B2 claim that polyoxyl-15-hydroxystearate can also be used as an effective solubiliser and stabiliser in prostaglandins formulations. Presented data revealed formulation superiority in terms of the results of stability testing, with reduced active substance degradation in storage than in the case of the reference and other surfactants used in ophthalmic preparations (Haut and Mercier, 2014).

There are also other substances such as cyclodextrins (CD) that can be efficiently used as solubility enhancers (Brewster and Loftsson, 2007). These are cyclic oligosaccharides with six (αCD), seven (βCD), eight (γCD) or more α-D-glucopyranose molecules. Other derivatives with substituted units are also available, e.g. sugammadex (modified γCD), delivered intravenously for the reversal of rocuronium-induced muscular blockade (Loftsson, 2021). In general, cyclodextrins are used as additives for the formulation with poorly soluble active substances in almost every delivery route. The European Medicine Agency (EMA) released a guideline for the use of cyclodextrins in drug formulation;

hydroxypropyl-β-cyclodextrin HP βCD is recommended for ophthalmics (European Medicines Agency, 2014), which is in line with research data (Loftssona and Järvinen, 1999).

Due to their lipophilic interior and hydrophilic exterior, cyclodextrins increase the solubility of many poorly soluble or insoluble drug substances (Loftsson and Masson, 2001; Stella, Valentino and Rajewski, Roger, 1997). This property fits perfectly with the lipophilic character of prostaglandin analogues; for example, latanoprost is dissolved and stabilised in the inclusion complex with cyclodextrin. The nature and ability of cyclodextrins to form dynamic inclusion complexes was successfully applied in latanoprost formulation, for which even better stability and ocular tolerance was described than for the reference (Xalatan) (Rodriguez-Aller et al., 2015).

Lipid emulsions are the next option for preparing preservative-free eye drops with prostaglandin analogues. It was shown that an oil-in-water emulsion consisting of a medium-chain fatty acid triglyceride with oils such as, for example, peanut oil, soybean oil or olive oil, can be successfully homogenised with a polyvinyl alcohol (PVA)/glycerin water solution to give a latanoprost emulsion possessing good storage properties at room temperature (Sakai et al., 2005).

Patent US9629852B2 describes the benzalkonium chloride-free (BAC-free) nano-emulsion with latanoprost as an alternative to the preserved product (Xalatan). In addition to water for injection and water-soluble salts, ingredients such as polyoxyl-15-hydroxystearate, castor oil and polyethylene glycol are applied to obtain a transparent liquid emulsion with a particle size distribution below 100 nm. It was found to be stable and, more importantly, resistant to active ingredient (latanoprost) sorption on the proposed primary packaging material, namely low-density polyethylene (LDPE) (Halder et al., 2017).

As regards the containers and the closure systems for ophthalmic products, these are made of polymers such as low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polyethylene terephthalate (PET), cyclic olefin polymer (COP) or a mixture thereof. Owing to usability and quality factors, glass packaging is rarely used. The lipophilic nature of prostaglandin analogues brings into focus the issue of sorption – an interaction with the packaging materials or any other plastic components from the production line, such as sterilising filters. Depending on temperature, this unsought for quality change can rise to more than 5% after twelve months of storage at ambient conditions (Wong et al., 2006). It leads to active ingredient assay loss and mass imbalance with degradation products. Thus, overages are common in prostaglandin analogue formulations, as in the case of. Latanoprost/Timolol "AET" eye drop solution (Public Assessment Report – procedure number: DK/H/1894/001/DC).

On the other hand, more exhaustive studies have shown that surfactants play an important role not only in improved corneal permeability of prostaglandins, but also in decreased sorption phenomena. Studies on their nature and structure revealed the important role played by the "lipophilic tail" and the "hydrophilic head". It was found that surfactants with a hydrophilic-lipophilic balance (HLB) above 15 gave better protection against sorption on packaging material polymers (Jiao, 2008; Ochiai et al., 2012).

4.2. Viscosity enhancers and mucoadhesive agents

The polymers used in ophthalmic formulations also offer a great opportunity for developments in ophthalmics. In order to extend contact and delivery time, they can be applied not only to prostaglandin formulations but also, given the varied nature of their properties, to other conditions such as allergies, inflammations and Dry Eye Disease (DED). Hyaluronic acid and its salts (HA), carbomers, polyvinyl alcohol (PVA), and cellulose derivatives like hydroxyethyl cellulose (HEC) and hydroxypropyl methylcellulose (HPMC) are the most frequently applied.

Polymers have excellent properties; hyaluronic acid (HA), for example, is a substance naturally occurring in ocular tissues that is perfectly safe and has excellent biocompatibility characteristics (Becker et al., 2009). It can be introduced to speed up corneal wound healing (Carlson et al., 2018),

supplement tear secretion in DED, and modulate ocular inflammation processes (Shoari et al., 2021), which are the primary triggers of the DED (Brignole et al., 2000; Stapleton et al., 2017). Moreover, cross-linked HA derivatives have been successfully applied to develop a sustained-release drug delivery system from liposomes. It was found that a modified HA cross-linked structure combined with liposomal encapsulated latanoprost significantly increased its release time (Widjaja et al., 2014). The studies referred to are an excellent step towards better compliance and efficacy regarding the physiological ocular barriers and poor drug availability after topical administration (Sasaki et al., 1996). Moreover, when combined with anti-inflammatories and tear substitutes, they can reduce the occurrence of side effects and therapy discontinuations, which are a particular challenge and of particular significance in chronic therapies.

For the same reasons, studies on ocular delivery are moving towards in situ gelling on the ocular surface. Substances such as pectin, trehalose, guar gum, chitosan and a few others are of special interest (Dubashynskaya et al., 2020). They are highly biocompatible, non-toxic and at the same time biodegradable, which are essential features in eye drop formulation. After topical administration, mucoadhesive polymers undergo a change in their structure towards sustained drug substance release (SR) systems. This can be induced by adjusting either the ionic strength, the temperature or the pH. An excellent example of this application is the chitosan-based and preservative-free formulation of latanoprost, where temperature was successfully applied as the driving force for SR formation. In the rabbit glaucoma model, it was found that the chitosan hydrogel formulation with latanoprost administered once weekly was as effective in lowering IOP as the reference once-daily treatment with Xalatan (Cheng et al., 2016). For the same reasons, synthetic substances such as poloxamers and acrylic acid derivatives became helpful in the *in situ* gelling in ocular formulation development. Specifically, in the case of poloxamer it was the temperature (Soliman et al., 2019) and in the case of acrylic acid the pH (Rupenthal et al., 2011) that was adjusted to achieve successful SR drug delivery. In general, formulations based on this principle ensure longer ocular contact time with less irritation and ocular drainage, and thus increased bioavailability. As a result, this approach offers an excellent alternative for chronic treatment with preserved eye drops or with invasive treatments such as implants.

In chronic treatment, patient discipline and convenience play an essential role in therapy success. Discontinuation in glaucoma therapy, due to its "asymptomatic nature" – the lack of symptoms, is quite significant (Beckers et al., 2008). Additionally, the use of preservative additives in ophthalmics and the adverse effects related to their use, e.g. DED, increase in the number of patient dropouts (Baudouin et al., 2010). Therefore, based on a patient-centred design approach, in order to improve patient compliance and therapy adherence, sustained-release delivery systems play a key role in glaucoma treatment. In 2020, the first biodegradable bimatoprost implant was approved by the U.S. Food and Drug Administration (FDA) (Seal et al., 2019; Shirley, 2020). In the phase 3 study, it was found to be effective in lowering IOP and not at all inferior to timolol therapy (Medeiros et al., 2020). The inventors used Hot Melt Extrusion (HME) technology (Ren et al., 2019) for the implant manufacture. In addition to 10 µg bimatoprost, it consists of poly(D,L-lactide), poly(D,L-lactide) acid-terminated, poly(D,L-lactide-co-glycolide) and polyethylene glycol 3350 ("Full prescribing information DURYSTA (bimatoprost intracameral implant)," 2001). Moreover, it can be sterilised by irradiation, which makes the process convenient and safe in comparison to aseptic processing. Although the implantation procedure does have its limitations and has to be performed by experienced personnel, the polymer structure of the implant is neutral to the ocular tissues. After administration, its matrix is metabolised to lactic and glycolic acids, carbon dioxide and water (Lee et al., 2010). The IOP-lowering effect lasts 4 to 6 months, which is of considerable benefit, compared to the standard eye-drop treatment, for patients who cannot manage with topical therapies.

Given that implantation is an invasive procedure, there are other solutions, such as therapeutic contact lenses (TCL), that will permit successful drug delivery without direct interference with the eye tissue barriers. As drug bioavailability after eye-drop delivery is poor (Zhang et al., 2004), TCLs afford an

excellent opportunity for the creation of a sustained-release system. In fact, such systems would seem to be especially convenient in conditions that require frequent eye-drop delivery, such as DED, or in the case of disorders, such as glaucoma, that call for chronic treatment.

There are a few methods for producing such a dosage system. The first relies on the diffusion process, in which the contact lens is soaked in a solution of the drug substance; then, after placement, the saturated TCL releases the absorbed substance. The second method uses matrix formation in which TCL polymers are mixed with colloidal micro- and nanoparticles containing an active substance. The active substance is then released once the lens matrix is at a specific pH or temperature, or has been adjusted to a specific ionic strength. The third method is to cover the lenses with polymers in which the active substance is dissolved, or the lens can be loaded with polymeric implants, e.g. on the external surface, without affecting vision in the central portion (Musgrave and Fang, 2019).

A wide range of polymer properties allows for the development of an almost infinite variety of release models that will guarantee success in specific therapy conditions (Choi and Kim, 2018). The importance of delivery systems involving wearables, such as contact lenses, should increase with time. There are numerous studies targeting issues relating to polymer use versus vision influence and manufacturing costs. Once such issues have been resolved, it will clear the way for a broader approach to the question of therapy (Musgrave and Fang, 2019).

4.3. Nanotechnology in glaucoma eye-drop formulation

The bioavailability of eye drops is very low, estimated to be 5% of the dose (Juliana, Fidiniaina et al., 2019), though attempts to improve bioavailability have been made. Nanotechnology is one of the approaches to increasing bioavailability and reducing side effects at the same time. Nanotechnology is focused on introduction of nanosized active pharmaceutical ingredient systems through the tissues. Nanosystems used in glaucoma treatment are composed of natural or synthetic polymeric materials. It ensures controlled release, low eye irritation, improved drug bioavailability and enhanced ocular tissue compatibility. A scheme showing the different nanotechnology-based components of an ocular delivery system is presented in Figure 3.

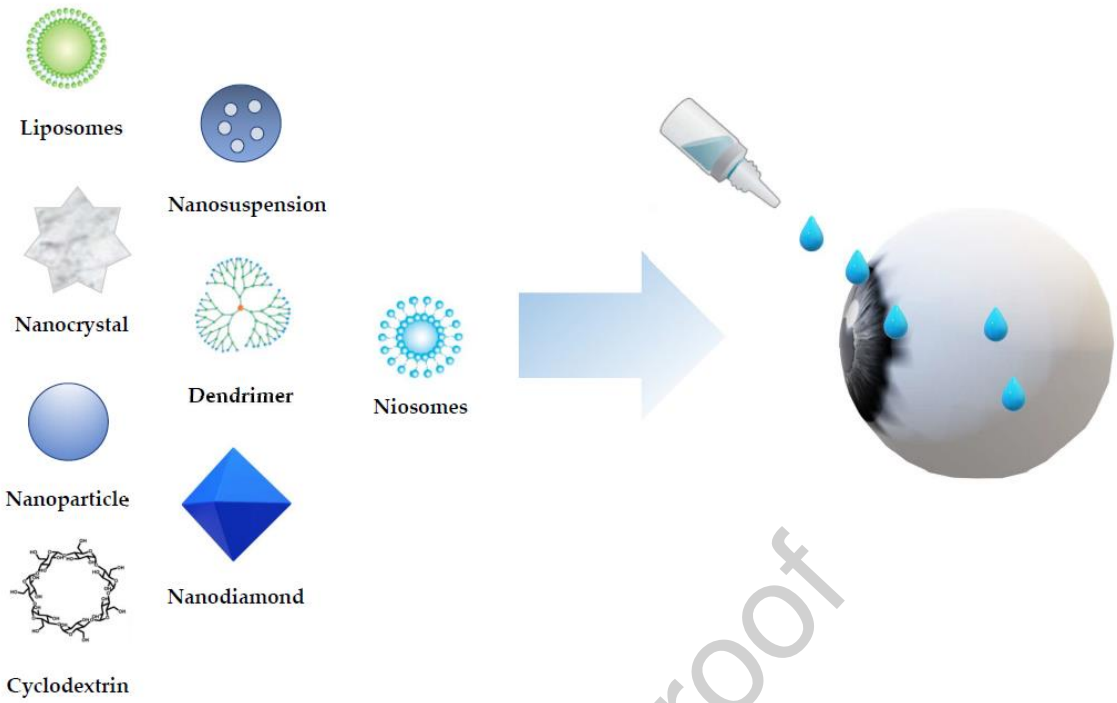


Figure 3. *Schematic illustration of different nanosystems used as ocular delivery systems* (Kwon et al., 2020).

Nanomaterials can incorporate drugs by encapsulation or conjugation. The encapsulated drug is released when the nanomaterial degrades at the target site. A drug conjugated to a nanomaterial is released when the bond between the nanosystem and the drug is cut at the target site. The drug type (hydrophobicity, stability, size), target tissue and route of administration determine the selection of an appropriate nanosystem. A few approaches have been described in the literature related to latanoprost therapy based on nanomaterials. Based on liposomes, nanosystems for sustained latanoprost delivery to the target site were developed. Both the latanoprost-loaded liposomes and the latanoprost/thymoquinone-loaded liposomes showed sustained drug release and lowered the IOP in glaucomatous white albino rabbits. The maximum IOP lowering effect lasted up to 84 hours (Fahmy et al., 2018). In another example, latanoprost-loaded EggPC liposomes were administered by subconjunctival injection and tested for IOP reduction in a rabbit glaucoma model and compared to Xalatan (commercial latanoprost eye drop) therapy. The therapeutic effect was comparable: Xalatan eye drops vs. latanoprost-loaded EggPC liposomes up to 120 days after first injection gave similar results in IOP reduction. A second injection of latanoprost-loaded liposomes reduced the IOP over the subsequent 180 days (Natarajan et al., 2014).

Niosomal gel – a gel incorporating niosomes – serves as a novel latanoprost nano delivery system. When the gel was tested in the eyes of rabbits, it showed a sustained drug release resulting in IOP reduction over a period of 3 days (Fathalla et al., 2020).

Cyclodextrins are used for nanosystems due to their ability to enhance the solubility of lipophilic drugs by forming inclusion complexes (Liu et al., 2016). Latanoprost-γ-cyclodextrin microaggregate suspension eye drops showed a sustained release and improved stability compared to Xalatan. *In vivo* studies in rabbits showed that latanoprost-γ-cyclodextrin eye drops exhibit higher bioavailability and a longer precorneal retention time (Zhou et al., 2021).

Another example of a latanoprost nanoparticle is a combination of hyaluronic acid, chitosan and latanoprost link nanoparticle (HA-CS-latanoprost link NP) formulated eye drops. CS-latanoprost link NP were obtained by the ionic gelation method. HA was adsorbed onto the exterior of positively charged

CS-NPs. This combination helps to achieve a high mucoadhesive effect, resulting in a higher drug concentration in a controlled manner. Experiments performed in rabbits have shown that the mean daily IOP-lowering effect was highest with the HA-CS-latanoprost link NP combination. The peak effect occurred after six hours, giving an IOP reduction of 4.85 mm Hg (37%) with plain latanoprost and of 4.8 mm Hg (36%) with Xalatan. With the HA-CS-latanoprost link NP the effect after six hours was a reduction of 5.75 mm Hg (43%) (Rubenicia et al., 2021).

4.4. Preservatives in ophthalmic formulation

As most ophthalmic products are presented in multi-dose containers, with a stated in-use shelf life after first opening, the need to use antimicrobial agents becomes a critical feature of the ophthalmic product. Compendial standards also require the use of preservatives in multi-dose products. These requirements are in line with quality and patient safety standards, as microbiological contamination can cause a drug to lose its potency and its properties, or it can be a source of infection. Table 8 presents a list of preservatives commonly used in ophthalmic formulation, together with their action mechanism and side effects.

Table 8. *Preservatives in ophthalmic formulation.*

Preservative	Brief description and mechanism of action	Adverse effects
Benzalkonium chloride (BAK)	The cationic surfactant – a quaternary ammonium compound used at a concentration of 0.004-0.025%. It is effective against gram-positive as well as gram-negative bacteria. The addition of ethylenediaminetetraacetic acid (EDTA) as the chelating agent enhances BAK activity against fungi. Its mechanism of action is based on dual nature - hydrophobic/hydrophilic properties. Thanks to this feature, it acts as a surfactant and interferes with lipid components in the bacterial cell membrane, resulting in cell disintegration (Baudouin et al., 2010; Freeman and Kahook, 2009; Steven et al., 2018).	Tear film instability, corneal damage, stinging and foreign body sensation are the most frequently reported adverse reactions associated with BAK use. Moreover, it has been found to be toxic to epithelial corneal cells. Causing inflammatory cascade, BAK is associated with DED development. European Medicines Agency (EMA) recommends that the use of this preservative should be avoided or at least limited, if possible (Freeman and Kahook, 2009; Jaenen et al., 2007).
Polyquaternium-1 (PQ-1)	A cationic surfactant – a quaternary ammonium compound – used in a concentration of about 0.001%. Compared to BAK, it has a smaller hydrophobic domain but far greater molecular weight. Current studies suggest that it is too large to enter mammalian cells, and therefore less toxic and safer than BAK. Its mechanism of action involves the destruction of cytoplasmic membrane integrity and, eventually, cell disintegration (Brignole-Baudouin et al., 2011; Codling et al., 2003, 2005; Rolando et al., 2011).	Though less cytotoxic than BAK, it has been reported to be more involved in the inflammatory reaction triggered on the corneal surface (Paimela et al., 2012) that can lead to DED development in case of prolonged use.
Cetrimide	A cationic surfactant and a mixture of three quaternary ammonium bromide salts: tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide. It has bactericidal activity. Its mechanism of action is similar to that of BAK and is based on its surfactant properties. Cetrimide affects cell barrier function.	Due to its mechanism of action, cetrimide contributes to tear film instability and deterioration in ocular surface condition. It is therefore not recommended for use in DED patients undergoing chronic treatment (Debbasch et al., 2001).
Thiomersal (or thiomerosal)	Ethyl mercuri-thiosalicylate, active against bacteria and fungi at a concentration of 0.01 to 0.001%.	It often causes allergic reactions. Currently, it is not used because it has been confirmed to cause

	It alters calcium homeostasis and, consequently, any calcium-dependent process in cells. Thiomersal inhibits cell cycle progression (Elferink, 1999; van Horn et al., 1977).	damage to the central nervous system (Elferink, 1999; Epstein et al., 2009).
Sodium perborate	Sodium perborate is oxidative preservative that is effective against a wide range of bacteria and fungi. When mixed with water, sodium perborate release hydrogen peroxide which acts by suppressing protein synthesis in microorganism cells. Once delivered to the eye surface, it is broken down into oxygen and water by enzymes (Freeman and Kahook, 2009; Noecker, 2001).	Due to its mechanism of oxidative DNA damage, it may be linked with a mutagenic effect. However, some reports suggest that it is safe at concentrations used in ophthalmic formulations. It is known to cause a stinging feeling (Noecker, 2001; Seiler, 1989).
Stabilised Oxychloro Complex (SOC)	Although it is a combination of chlorine compounds (chlorite, chlorate, chlorine dioxide), it acts as an oxidising rather than a chlorine agent. Its mechanism of action is based on chlorine dioxide radicals which oxidise cell membrane components – unsaturated lipids (Noecker, 2001).	Due to its oxidative activity, SOC can cause damage to ocular epithelium, including punctuate epithelial erosions. Although considered safe, it is not recommended for chronic treatment (Dutescu et al., 2017; Schrage et al., 2012).
Polyhexamethylene biguanide (PHMB)	This biguanide is a cationic compound which binds to the negatively-charged phospholipids in cell walls and membranes of bacteria. It affects membrane permeability by changing barrier function; it also binds to bacterial DNA and interferes with bacterial growth. This dual mechanism of action results in cell death. When used at low concentrations, e.g. from 10 up to 600 µg/ml, it was found to be effective against pathogenic bacterial strains, but less effective against fungi. In combination with chlorhexidine. it is recommended for the treatment of Acanthamoeba keratitis (Chindera et al., 2016; Ferrari et al., 2011; Zhi et al., 2017).	Undiluted PHMD caused irreversible changes in the rabbit eye in mucosal and eye irritation tests. However, PHMB at low concentrations (up to 100 µg/ml) is considered safe and was found to be non-toxic to human corneal epithelial cells (SCCS, 2015; Yanai et al., 2006).
Chlorobutanol	Chlorobutanol disrupts the lipid structure of the cell membrane, leading to cell lysis (Noecker, 2001).	It is known to cause eye irritation in more than 50% of patients, most likely due to influence on cell-division cycle in corneal epithelium. Chlorobutanol was shown to induce the loss of corneal epithelium layers and keratitis (Noecker, 2001).
Methylparaben	Methylparaben is more active against moulds and yeasts than bacteria. Its mode of action is based on its influence on membrane transport and disruption of mitochondrial processes (Soni et al., 2002).	It causes eye irritation. In a number of studies corneal cell damage, loss of goblet cells and keratinisation were observed (Soni et al., 2002).
Sorbic acid	Sorbic acid influences cell membrane and transport functions; additionally it causes proton flux into cell. It is active against both vegetative forms and spore germination (Sofos et al., 1986).	It causes eye irritation, punctate keratitis (Kaur et al., 2009; SCCS, 2015).
Ag ions	As positively charged ions, Ag ions have an affinity for sulfur-containing proteins. They adhere to various cell membranes affecting their permeability. Inside the cell, silver ions can deactivate enzymes responsible for cell respiratory processes and ATP formation. Adherence to sulfur and phosphorus ions affects replication of DNA	Patient sensitivity to silver ions results in adverse effects such as allergies. Moreover, As ions can interfere with drug composition (Hadrup et al., 2018).

	replication and protein synthesis (Durán et al., 2016; Khorrami et al., 2018)	

According to recent reports (Baudouin et al., 2010; Holló et al., 2018), despite being very effective in combating pathogens, preservatives have not been designed to be neutral to the eyes. By inhibiting bacterial growth, they also damage corneal and conjunctival epithelial cells, especially in the case of long-term DED, for example, or in chronic use for the treatment of glaucoma. In the vast majority of preserved products, BAK is used as the antimicrobial agent. Reports indicate that about 70% of eye drops are preserved with this substance (Freeman and Kahook, 2009; Steven et al., 2018). Recent years have proved that therapy discontinuation due to preservative intolerance plays an important part in patient compliance and therapy adherence (Holló et al., 2018; Tang et al., 2019). Extensive research has shown that long-term use of eye drops containing preservatives has a negative effect on the eye, and glaucoma patients are taking such medicines for the rest of their lives. It is also commonly known that patients with this or any other chronic ocular disease treated with preserved eye drops suffer from undesirable effects such as irritation, allergic reaction and, in extreme cases, inflammation. Moreover, DED induced by chronic preservative use has been reported as one of the serious conditions preventing effective therapy (Freeman and Kahook, 2009; Jones et al., 2017). Therefore, limiting the use of preservatives and eventually eliminating them altogether from eye-drop formulations forms the basis of the modern approach to ophthalmic/glaucoma product development ("Quality data requirements to demonstrate suitability of multidose containers for preservative free eye drops NEW October 2018," 2018).

It used to be claimed that BAK played an important role in drug substance permeability and efficacy. Comparative clinical trials on preservative-free versus preserved products has, however, shown the opposite to be true – there is no difference in efficacy in terms of IOP control (Holló et al., 2018; Thygesen, 2018). The advantage of preservative-free glaucoma drops is significant: they definitely give rise to fewer adverse reactions as well as fewer therapy discontinuations (Misiuk-Hojlo et al., 2019). Effective/compliant therapy ensures that cases are less severe and the cost to the health care system is reduced (Garrigue et al., 2017).

The features described have been taken into account in the development of customised packaging systems designed to provide preservative-free eye-drop delivery. Single-dose polyethylene containers, called minims, were the natural choice. The first products to come onto the market appeared in the 1960s. However, it was, and still is, a less than perfect solution. The uncontrolled manner of opening increases the risk of damaging the eye surface during delivery. Secondly, water loss from the small-volume semi-permeable container requires that it be stored in additional barrier packaging. Owing to their size, minims are easy to lose; even after opening, there is still a temptation to reuse them; moreover, they tend to generate a considerable amount of waste. Additionally, the size of the single-dose packaging has a negative influence on the drug's usability – elderly patients with reduced manual dexterity have difficulty in handling them. Finally, they are more expensive than those in multi-dose packaging systems for preserved and unpreserved eye drops. All the above reasons are the main drawbacks to the use of the single-dose containers.

Microbiological safety of the ophthalmic drug is the primary requirement. If ignored, it would influence not only drug quality but, in particular, patient health. However, there are ophthalmics available in specially designed containers that allow preservative-free products to be delivered conveniently and effectively. Among others on the market, there are the ABAK® system developed by Théa Laboratories, the COMOD® dosage system from Ursapharm, Nemera's NOVELIA® multidose eyedropper and the 3K® Multidose System from AeroPump. These systems are equipped with features that guarantee the microbiological safety of the container content and the microbiological quality of the delivered drops. Depending on the system's construction, essential features relate to the air filter, the one-way valve, and

antimicrobial agent such as silver ions. Filtration of every delivered drop is an additional option with these systems.

The EMA has recommended a series of measures designed to ensure the safety of preservative-free ophthalmic drugs. Particular emphasis is placed on in-use stability testing as this is the area where there is the greatest risk of patient error and loss of drug microbiological quality. These consist of a series of tests simulating severe patient misuse, in which the one-way valve and, if present, silver ion activity are tested with the aid of so-called bacterial challenge tests. High concentrations of pathogens such as *P. aeruginosa, S. aureus, E. coli, C. albicans, A. brasiliensis* are applied periodically to the dropper tip throughout the whole declared in-use period. It is assumed that the contamination originates from accidental touching with the finger, cheek or eyelid. In other words, the dropper tip is contaminated by swabs exposed to a particular pathogen inoculum. In contrast, venting filter performance is evaluated in an air environment contaminated with *B. diminuta*. Being one of the smallest of microorganisms, it is specially recommended for testing filter performance and filter integrity. As in the dropper tip challenge, this test is performed with use simulation of the expected time and manner of product delivery. The microbiological activity of the silver ion is tested in accordance with the compendial requirements (Ph. Eur. 5.1.3. Efficacy of Antimicrobial Preservation). In this test, the residual drop volume versus silver element surface is the determinant test space. The microbial activity of the released ions is measured by the growth reduction defined by the compendial requirements. In each of the above tests, the sterility of the packaging contents and the microbiological quality of the delivered drops are the critical experimental endpoints, and the three dimensions described provide proof of the system's robustness with regard to the microbiological safety of the product.

5. PHARMACEUTICAL ANALYSIS OF PROSTAGLANDIN ANALOGUES

The manufacturer of a medicinal product is responsible for providing a high quality product in accordance with the requirements of the current pharmacopoeia, pharmaceutical legislation, GMP rules and ICH guidelines appropriate to the type and form of the medicine. Quality assurance of the medicinal product relates to controlling the drug substance and excipients used in the manufacturing process and controlling the manufacturing process as well as the finished product, in compliance with the requirements included in the product specification (*USP NF. Ophtalmic products - quality tests.*, 2021).

This part of the paper focuses mainly on analytical issues related to determination of the content and quantification of related substances in prostaglandin analogues commonly used in the treatment of glaucoma, i.e. latanoprost, travoprost, bimatoprost and tafluprost, in the raw materials and pharmaceutical formulations, as well as in cosmetics. Of the PGAs used in the treatment of glaucoma, latanoprost and travoprost are pro-drug analogues of the isopropyl ester of 17-phenyl-$PGF_{2\alpha}$, while bimatoprost is an amide pro-drug of 17-phenyl-$PGF_{2\alpha}$. Tafluprost is a unique analogue of $PGF_{2\alpha}$ due to the replacement of the C-15 hydrogen and hydroxyl group with two fluorine atoms (Cai et al., 2021). The chemical structures of the described substances are presented in Figure 5.

When considering the analysis of pure substances intended for pharmaceutical use, several important analytical aspects of this group of compounds should be noted. The most important is that, to date, bimatoprost and tafluprost have not been officially listed in any major pharmacopoeia, such the United States Pharmacopeia (USP), the European Pharmacopoeia (Ph. Eur.), the Japanese Pharmacopeia (JP) or the British Pharmacopoeia (Abd-AlGhafar et al., 2020). Latanoprost is described in official monograph USP NF and Ph. Eur. The monograph of latanoprost was first published in the Ph. Eur. in Supplement 10.3 (01/2021:2230) and is so far the only official Ph. Eur. monograph for substances belonging to the group of prostaglandin $PGF_{2\alpha}$ analogues with anti-glaucoma activity. Travoprost is also officially described in two monographs of the United States Pharmacopeia, Travoprost and Travoprost Ophthalmic Solution.

The lack of any official analytical methods and of a specific quality standard for the drug substances including bimatoprost and travoprost place the responsibility for assessing the chemical quality and safety of the substances on the manufacturer of the active substances and on the manufacturer of the finished product, based on the available scientific knowledge (ICH Q3A: Impurities in New Drug Substances (Agency, 2006) ICH Q3B: Impurities in New Drug Products guideline (European Medicines Agency, 2006) and on experimental studies.

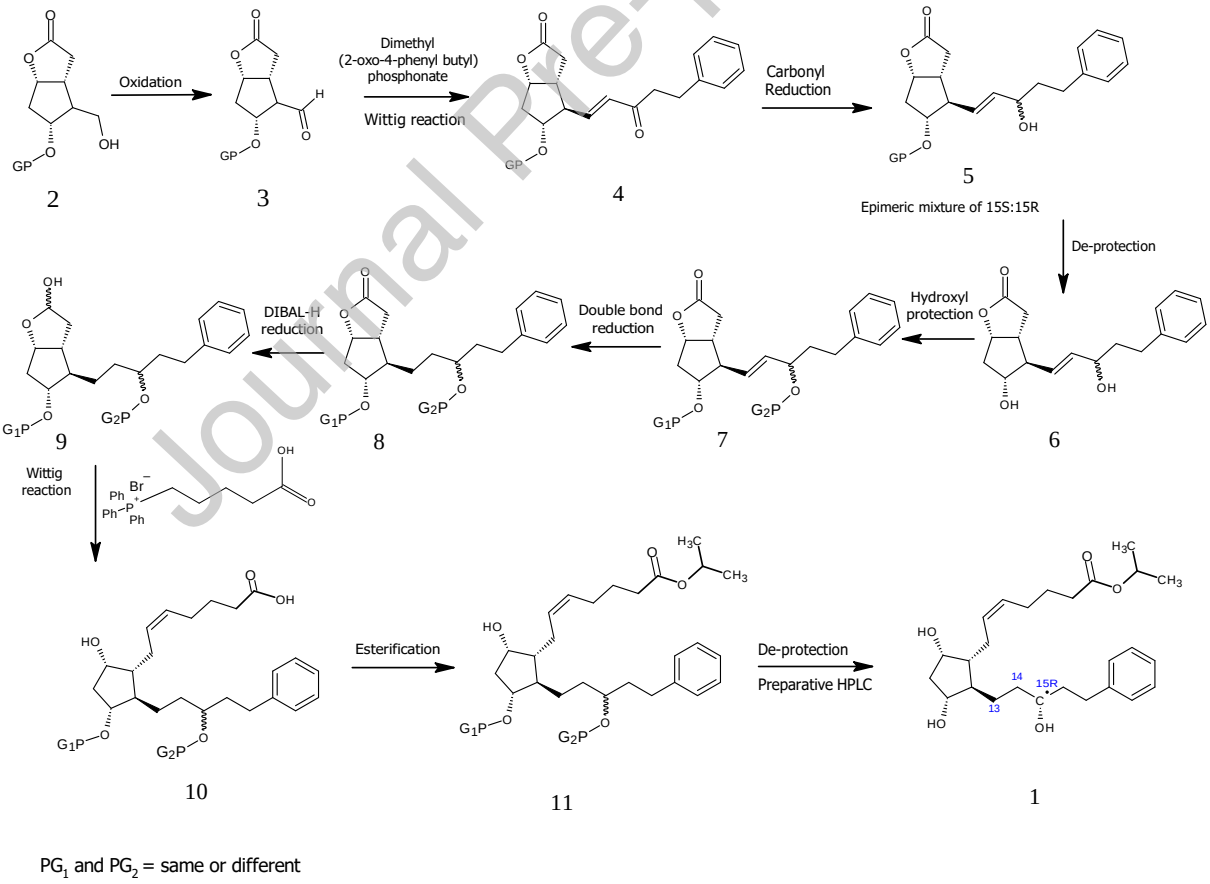
A very important issue in the evaluation of related substances of prostaglandin analogues is the phenomenon of optical and geometric isomerism. The chemical structures of the mentioned PGF analogues are very similar. They are characterised by two hydroxyl groups in *cis* configuration relative to the cyclopentane ring and two side chains α and ω in trans configuration relative to each other. The α chain contains an aromatic group and the other ω chain contains a carbonyl functional group. The double bond in the α chain between the C5 and C6 carbon atoms in the *Z* configuration is a common feature of pharmaceutically-active $PGF_{2\alpha}$ analogues, whereas the double bond in the ω chain may be absent, as it is in the latanoprost molecule (Albert, 2010). In addition, the bimatoprost, travoprost and latanoprost molecules possess 5 chiral centres, and tafluprost one, less in due to the C-15 carbon being replaced by 2 fluorine atoms. Latanoprost and travoprost molecules have the *R* configuration on the C-15 carbon, in contrast to bimatoprost, which has the opposite *S* configuration on the C-15 carbon. The multiple chiral centres and a few double bonds present in these structures yield potentially a large number of isomers that should be regarded as potential impurities of the substance. In the interests of pharmacotherapeutic safety, the very high potency of prostaglandins (Martynow et al., 2007) demands very strict control of impurities, especially impurities of isomers of unknown activity.

The presence of diastereoisomers in a pure substance is related to organic synthesis based on the classical Corey method, which consists in the sequential attachment of α and ω side chains to a commercially available Corey aldehyde/lactone derivative (Dams et al., 2013a). Scheme 1 (Sasane et al., 2019) shows the synthesis of latanoprost by the classical Corey method. A limitation of Corey's strategy is the lack of stereoselectivity of the reduction of ketone function of C-15, providing mixtures of 15R/15S epimers in a ratio dependent on the reactant used and reaction conditions. In practice, it is not possible to obtain 15-OH derivatives of a strictly defined C-15 configuration with selectivity clearly exceeding 99%. Due to the very similar physicochemical properties of the 15R/15S epimers, removal of significant amounts of the undesired 15-epi isomer is possible only by using tedious and multi-step purification procedures (Dams et al., 2013a). However, continuous improvements in the synthesis of prostaglandin analogues, with the introduction of more efficient methods for the purification of intermediates and finished products, as well as modifications of the classical synthesis route, have led to a significant reduction in the amount of undesired diastereomers.

Martynow *et al*. in 2011 (Martynow et al., 2007) proposed a new method also from Corey lactone starting material, using prostaglandin phenylsulfone intermediate, in which they obtained only trace amounts of 15(*S*)-latanoprost. In 2013, Dams *et al*. (Dams et al., 2013a) also used the above-mentioned phenylsulfone to synthesise travoprost and bimatoprost, obtaining bulk drug with trace amounts of epimers (0.18% of 15-(*S*)-travoprost and 0.12% of 15-(*R*)-bimatoprost). In 2019, Sasane *et al.* (Sasane et al., 2019) experimentally confirmed that, in the classical Corey method leading to latanoprost, 15(*S*)-latanoprost can be formed during the reduction of a carbonyl ketone intermediate (chemical compound **4** in Scheme 1) and by inversion at the chiral C-15 due to the presence of allyl groups at the C13,14 position in the diol (**6**) in subsequent synthesis reactions. The authors therefore introduced an improved process related to US2009/025906 (Costantino, Francesca , Di Brisco, 2010), which replaced the previously used purification of carbonyl ketone intermediate (compound **4** in Scheme 1) by column chromatography with a much more efficient crystallisation and modifying the synthesis by the use of diol intermediate for double-bond reduction prior to hydroxyl protection. The content of 15(*S*)-latanoprost can vary significantly depending on the synthesis strategy adopted. As demonstrated by Sasane *et al*. (Sasane et al., 2019), latanoprost obtained by the method described in patent

US2009/025906 contained 3.15% of 15-(*S*) diastereomer, whereas latanoprost produced by the same method, but using enantiomerically-pure diol (compound **6** in Scheme 1) (de 99.88%) contained only 1.82% of the unwanted isomer. In contrast, the modification by Sasane *et al*. (Sasane et al., 2019) yielded latanoprost comprising only trace amounts of the diastereomer at 0.05%.

The second typical impurity that formulation cannot avoid during the synthesis of latanoprost using Corey's lactone is 5,6-trans latanoprost. In the final steps of the synthesis, Martynow *et al*. (Martynow et al., 2007) observed formation of a small amount (around 5-9%) of the 5,6-trans isomer of latanoprost which, however, was readily eliminated from latanoprost by preparative HPLC on silica gel stationary phases and gave latanoprost of greater than 99.9% purity. In European Patent EP 2 208 724 A1 (Costantino, Francesca , Di Brisco, 2010) it is also noted that in several different modifications of the Corey method impurities can be formed, including three isomers of latanoprost, the 15(*S*)-5,6-cis isomer, the 15(*R*)-5,6-trans isomer and the 15(*S*)-5,6-trans isomer. In EP 2 208 724 A1 (Costantino, Francesca , Di Brisco, 2010) the authors described the new purification process that led to pure latanoprost with a content of each of the above three isomers below 0.1% being obtained. Furthermore, US Patent 2014/0051882 (TSAI, 2014) A1 lists impurities such as 5,6-*trans*-tafluprost, 5,6-*trans*-travoprost, 16(*E*)-1F-tafluprost and 16(*Z*)-1F-tafluprost, which are also formed as synthetic impurities. The content of 5,6-*trans*-tafluprost or 5,6-*trans*-travoprost can be reduced to less than 0.5% after purification using preparative HPLC, while impurity 16(*Z*)-1F-tafluprost can be completely eliminated (TSAI, 2014). Despite the development of increasingly pure substances, quality control of substances for pharmaceutical use must be carried out on the basis of highly sensitive and stereoselective analytical methods, because the presence of undesirable chemicals – even in small amounts – may influence not only therapeutic efficacy but also the safety of the pharmaceutical product (Mehta et al., 2010).



Scheme 1. *Classical Corey route of synthesis for latanoprost* (Sasane et al., 2019).

In general, prostaglandins have low water solubility and are unstable. They are prone to oxidation and to hydrolytic, thermal and photochemical degradation. Velpandial *et al.* (Velpandian et al., 2015) confirmed the high degree of degradation of latanoprost in methanolic pure solution at a concentration of 7 µg/ml by testing the effects of acid (HCl 5M) and base (NaOH 5M), thermal effects at 40°C for 48 hours, hydrogen peroxide-induced oxidation at 30% v/v and photodegradation by 24 hours' exposure at room temperature (25°C). Forced degradation studies showed 91 and 95% degradation in both acidic and basic conditions, respectively, which was found to be statistically significant ($p < 0.001$) compared to the control. Hydrogen peroxide at 30% concentration degraded latanoprost by as much as 20% over a period of 6 h, and the reduction was found to be statistically significant ($p < 0.05$) compared to the control. Latanoprost standard exhibited 13% degradation under white light ($p < 0.05$). Exposure to a temperature of 40°C led to 35% degradation ($p = 0.01$). In addition, Morgan *et al*. (Morgan et al., 2001) reported that latanoprost in the original ophthalmic formulation, Xalatan, remained stable at 4 and 25°C for the 30-day duration of the study. Analysis of concentration versus time curves for 50 and 70°C yielded a t90 (time for 10% degradation) of 8.25 and 1.32 days, respectively. Ultraviolet B radiation caused a rapid degradation of latanoprost, but ultraviolet A radiation was less effective. The author suggested that due to latanoprost thermal and solar instability, it should ideally be stored below room temperature and in the dark (Wittenberg et al., 2014). Similarly, Johnson *et al*. (Johnson et al., 2011) observed that latanoprost ophthalmic solution was stable at 27°C, but when stressed at 37°C or 50°C latanoprost degraded at a rate of 0.15 or 0.29 mg/ml/day, respectively. Under the same thermal stress condition, travoprost ophthalmic solution was stable at 27°C and 37°C, but with storage at 50°C travoprost degraded at a rate of 0.46 mg/ml/day. Bimatoprost remained stable for 30 days at 27°C, 37°C and 50°C (Wittenberg et al., 2014). It was also reported that latanoprost is a weakly UV-absorbing compound that only exhibits considerable absorbance in the middle-UV region (Wong et al., 2006). The above-mentioned characteristics introduce certain limitations in the selection of analytical techniques and make working with these substances in the laboratory quite difficult The main analytical challenges for the determination of the contents of latanoprost, travoprost and tafluprost and related substances in pharmaceutical formulations are the development of suitably highly sensitive procedures owing to the very low concentrations used in ophthalmic solutions i.e. 50 µg/ml, 40 µg/ml and 15 µg/ml, respectively. Only the concentration of bimatoprost used in eye drops is significantly higher and ranges from 0.1 mg/ml to 0.3 mg/ml. Bimatoprost at the 0.3 mg/ml concentration has also been officially used since 2008 to treat eyelash hypotrichosis, or sparse eyelash growth. It is the first PGF analogue to be approved only by the American Food and Drug Administration and not by the European Union for cosmetic purposes (Marchei et al., 2016). Special tests should be conducted on eyelash enhancing products, especially if sold on websites, to determine the presence of prostaglandin analogues that can cause side effects such as eye irritation, itching eye pain, change of eye colour and darker pigmentation around the eye (Marchei et al., 2016). Sample preparation is a crucial step in the analysis of cosmetics. Very complex matrices, whose composition can be completely different between two products used for the same purpose (Wittenberg et al., 2014), require the use of a suitable and efficient extraction technique prior to chromatographic analysis.

The high sensitivity of analogues to degradation when exposed to light, temperature (Johnson et al., 2011; Morgan et al., 2001) and oxidizing agents (Velpandian et al., 2015), as well as their ability to adsorb on packaging materials (Le Basle et al., 2017), especially in the case of latanoprost and travoprost may make it difficult to determine the correct impurity profile due to mass imbalance. If the measured increase in degradation products is less than the loss of API, the erroneous conclusion may be that the method is not suitable for the intended purpose, as it cannot detect and accurately determine all degradants. On the other hand, there is a need for vigilance and for the development of specific and suitably sensitive methods. The observed decrease in the substance content should not be attributed solely to the adsorption phenomenon. Furthermore, the diversity of pharmaceutical formulations, including the presence of a second active ingredient with synergistic effects but with completely

different physicochemical properties and at many times higher concentrations, also seems to be an issue that calls for further attention.

Detailed information is presented on latanoprost, travoprost and bimatoprost, the most commonly used prostaglandin analogues, with particular emphasis on related substances and products of degradation, with descriptions of compendial methods and other reported analytical methods using HPLC and UPLC techniques with UV detection.

The chemical structure of latanoprost is given as Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate (Elkady et al., 2018). The new European Pharmacopoeia latanoprost monograph describes 10 potential latanoprost-related substances named A, B, C, D, E, F, G, H and J, but only three of them, i.e. impurities E, F and H, require control in the drug substance, according to their own limit. Other listed identified impurities should be treated as any other impurity for which the maximum acceptable limit is 0.1% (Pharmacopoeia, 2021) . The chemical names of impurities D, E, F and H are shown in Table 10, while the structures of impurities E, F and H are shown in Figure 5. The chemical names of the other impurities are listed below in Table 9.

Table 9. *The chemical names of latanoprost impurities A, B, C, G and J.*

Latanoprost impurity	IUPAC chemical name
Impurity A	propan-2-yl(5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-2-[(3*R*)-3-(formyloxy)5-phenylpentyl]-3,5-di-dihydroxycyclopentyl]hept-5-enoate
Impurity B	propan-2-yl(5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3-(formyloxy)-5-hydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]-cyclopentyl]hept-5-enoate
Impurity C	propan-2-yl(5Z)-7-[(1*R*,2*R*,3*R*,5*S*)-5-(formyloxy)-3-hydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]-cyclopentyl]hept-5-enoate
Impurity G	methyl(5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*]-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]-cyclopentyl]hept-5-enoate
Impurity J	propan-2-yl(5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-2-[(3*R*)-5-phenyl-3-[(triethylsilyl)oxy]cyclopentyl]hept-5-enoate.

The requirements included in the official monographs are similar. However, in Ph. Eur. the limits for 15(*S*)-latanoprost and latanoprost acid are slightly lower than those in the USP monograph (USP-NF, 2021). A comparison of the nomenclatures and requirements for impurities of latanoprost included in both the USP and the European Pharmacopeia is presented in Table 10. The limits adopted for the latanoprost ophthalmic solution presented in the pending Latanoprost Ophthalmic Solution monograph result from the substance requirements and are set, respectively, for latanoprost compound A – 3.5% and for latanoprost compound B – 2.0%. In addition, according to the proposed monograph, three more impurities should be assessed in the product constituting a buffered solution of latanoprost (3*S*,*E*)-Latanoprost isomer (Isopropyl(*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*S*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate) and two impurities identified by their retention times, Impurity 1 (RRT ~0.16) and Impurity 2 (RRT ~0.22), with maximum permissible levels of 0.3%, 2% and 1%, respectively. The acceptable limit for total impurities has been established at 7% (Monograph, 2012).

Table 10. *Related substances of latanoprost API specified in Ph. Eur* (Pharmacopoeia, 2021) *and USP NF* (USP-NF, 2021).

Chemical name	Common name/abbreviation	USP nomenclature and acceptance criteria (NMT%)		European Pharmacopoeia nomenclature and acceptance criteria (NMT%)	
Isopropyl 5-(diphenylphosphoryl)pentanoate	IDPP	Isopropyl diphenylphosphoryl pentanoate	0.1%	Impurity D	-
Isopropyl (*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate	5,6-trans latanoprost	Latanoprost related compound A	3.5%	Impurity F	3.5%
Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*S*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate	(15S)-latanoprost	Latanoprost related compound B	0.5%	Impurity E	0.4%
(*Z*)-7-{(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl}-5-heptenoic acid	Latanoprost acid	Latanoprost related compound E	0.2%	Impurity H	0.15%
Any unspecified impurity	-	-	0.1%	-	0.10%
Total	-	Latanoprost related compound A and latanoprost related compound B are excluded	0.5%	Impurity F is excluded	1.0%

Latanoprost impurity F (5,6-*trans*-latanoprost) is the isomer of latanoprost with the double bond between carbon atoms 5 and 6 changed from *cis* (*Z*) to *trans* (*E*) in relation to latanoprost moiety (Widomski et al., 2008). Impurity F is related to organic synthesis. Latanoprost impurity E (15(*S*)-latanoprost) it is the isomer of latanoprost in which the hydroxyl group at carbon 15 is inverted relative to latanoprost (Widomski et al., 2008). It is a typical process impurity originating from organic synthesis (Dams et al., 2013a). Latanoprost impurity H (latanoprost acid) is the metabolite of latanoprost. Free acid is a pharmacologically active molecule 200-fold more active at the prostaglandin F_{2A} receptor than latanoprost. The octanol:water partition coefficient (logP) of free acid latanoprost is 0.52 (pH 7.4, 23.5°C). *In vivo*, it is formed very rapidly when latanoprost is hydrolysed by esterase enzymes, which are known to be abundant in the cornea (Sjöquist et al., 1999). Latanoprost acid is a known process impurity. Mehta *et al*. reported latanoprost acid as the major degradant under acidic conditions (5N hydrochloric acid, 6h, 40°C) during the forced degradation study of drug substance and drug product. Also, small amounts of this impurity were formed when the substance and product were exposed to elevated temperature i.e. 40°C for 48h and in a basic environment of 5N sodium hydroxide, for 4 minutes at 25°C (Mehta et al., 2010). Velpandian *et al*. (Velpandian et al., 2015) confirmed the presence of latanoprost acid in the original Xalatan product and in 6 generic variants of latanoprost formulations in controlled stress experiments. They also found latanoprost acid formation in the original product and 1 generic in a 30-day patient use study (Velpandian et al., 2015). 15-ketolatanoprost (9α,11α-dihydroxy-15-oxo-17-phenyl-18,19,20-trinor-prost-5(*Z*)-en-1-oicacid, isopropyl ester) is a degradation product of latanoprost as a result of oxidation (Mehta et al., 2010). The presence of small amounts of 15-ketolatanoprost was confirmed in all tested products in an experimental degradation study by Velpandian *et al*. (Velpandian et al., 2015), while in one formulation it was found at a higher concentration. Conflicting reports were presented by Mehta *et al*. (Mehta et al., 2010), who did not find 15-ketolatanoprost in the oxidised test, but reported that 0.42% and 0.53% 15-ketolatanoprost was determined when subjected to UV radiation (200 watt-hours m^{-2}) and heat stress condition (48h, 40°C), respectively. Velpandian *et al*. (Velpandian et al., 2015) also documented 15-keto latanoprost acid (7-(3,5-dihydroxy-2-(3-oxo-5-phenylpentyl)cyclopentyl)hept-5-enoic acid) as a degradation product after exposure of ophthalmic formulations to hydrogen peroxide. Moreover, the authors revealed that degradation studies showed the formation of three novel unknown impurities. The possible structures of two of them could be deciphered as Isopropyl 7-(3,5-dioxo-2-(3-oxo-5-phenylpentyl)cyclopentyl)hept-5-enoate (m/z 426.49) and Isopropyl 7-(3,5-dihydroxy-2-(3-hydroxy-5-phenylpentyl)cyclopentyl)hept-5-enoate (m/z 480.4), while the third remains unresolved. The concentration of impurities found in control degradation varied significantly in different generic formulations and Xalatan (Marchei et al., 2016).

Five identified and specified impurities should be controlled in travoprost ([1*R*-[1α(*Z*),2β(1*E*,3*R*),3α,5α]]-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[3-(trifluoromethyl)phenoxy]-1-butenyl]cyclopentyl]-5-heptenoic acid, 1-methyl-ethyl ester), according to the USP Pharmacopoeia. The chemical names and acceptable limits of impurities included in the travoprost monograph and monograph of Travoprost Ophthalmic Solution are presented in the Table 11. It is worth noting that the list of specified impurities for API is longer than in the latanoprost monograph and includes 15-ketotravoprost and epoxy derivative, in addition to related substances such as free acid, 15(*S*) isomer and 5,6-*trans* isomer (USP, 2021a)(USP, 2021b).

Table 11. *Related substances of travoprost API and Travoprost Ophthalmic Solutions specified in USP NF* (USP, 2021a, 2021b)

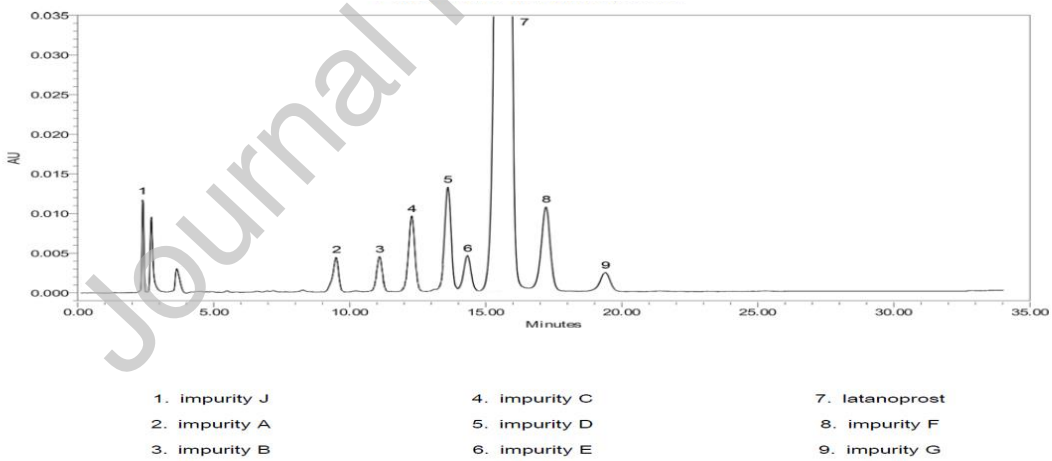
USP nomenclature	Chemical name	Acceptance criteria	
		API	Ophthalmic solution
Travoprost-related compound A (travoprost acid)	(5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(1*E*,3*R*)-3-hydroxy-4-[3-(trifluoromethyl)phenoxy]-1-butenyl]cyclopentyl]-5-heptenoic Acid	0.2%	1.0%
Epoxide derivative	(5*Z*)-(9*S*,11*R*,15*S*)-9,11,15-Trihydroxy-13,14-epoxy-16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-5-prostadienoic acid, isopropyl ester	0.4%	-
15-epi Diastereomer	(5*Z*,13*E*)-(9*S*,11*R*,15*S*)-9,11,15-Trihydroxy-16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester	0.1%	-
5-6 trans travoprost	Isopropyl (*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*R*,*E*)-3-hydroxy-4-[3-(trifluoromethyl)phenoxy]but-1-enyl]cyclopentyl]hept-5-enoate.	3.5%	5.0%
15-ketotravoprost	Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*)-3-oxo-4-[3-(trifluoromethyl)phenoxy]but-1-enyl]cyclopentyl]hept-5-enoate	0.3%	1.0%
Any other individual impurity	NA	0.1%	
Total	NA	4.0%	5.5%

Potential impurities of bimatoprost ([1*R*-[1α(*Z*),2β(1*E*,3*S*),3α,5α]]-7-[3,5-dihydroxy-2-(3-hydroxy-5-phenyl-1-pentenyl) cyclopentyl]-N-ethyl-5-heptenamide) are typical isomeric process impurities such as 15-epi-bimatoprost (15(*R*)-bimatoprost) and 5,6-trans bimatoprost. Impurities originating from degradation process 15-keto bimatoprost (Zezula et al., 2019). Bimatoprost acid, which is the result of amide hydrolysis and is considered an active moiety acting on FP receptors, is also regarded as an impurity (Faulkner et al., 2010). The chemical structures of process impurities and degradation products of latanoprost, travoprost, bimatoprost and tafluprost are shown in Figure 5.

The development of a specific, precise, accurate and stability-indicative method for the determination of content and related substances is essential in the development of a new drug product. Over the past 20 years, a large number of chromatographic methods with UV or MS/MS detection have been developed for the determination of prostaglandin analogues in crude material and in simple or complex pharmaceutical formulations. In Table 12 we summarise these methods, based on a reliable review of the available literature.

Most of the methods relate to the determination of latanoprost. The USP and Ph. Eur. (Pharmacopoeia, 2021; USP-NF, 2021) recommend determining the content of latanoprost and related substances, other than impurity H, by the normal phase HPLC method, with UV detection at 210 nm. The compendial methods are very similar. Both methods use normal phase chromatography on a silica column. The Ph. Eur. monograph uses the same column type under similar conditions (Pharmacopoeia, 2021), although the mobile phase contains heptane instead of hexane. Detailed information is presented in Table 12. Eight related substances named in accordance with the Ph. Eur monograph impurities A, B, C, D, E, F, G and J are well separated and can be quantified on the chromatogram provided by the EDQM (Pharmacopoeia, 2021). The official method described in the Ph. Eur. monograph is the one that presents the largest number of separate latanoprost-related substances.

Figure 4. *Chromatogram for the test for related substances of latanoprost by official Ph. Eur. method provided by the EDQM.*



According to the official monographs published in the Ph. Eur. (Pharmacopoeia, 2021) and USP (USP-NF, 2021), the latanoprost acid content should be determined by the reverse-phase HPLC method in the gradient mode, where the mobile phases are mixtures of phosphoric acid, acetonitrile and water in different proportions by volume and the stationary phase is a base-deactivated end-capped octadecyl silica gel for chromatography. The reverse-phase method can be utilised because the lipophilicity of imp. H is 7000 times lower than latanoprost (Sjöquist et al., 1999).

The first HPLC analytical method employing an NH_2 column for the determination of isomer 5,6-trans latanoprost and 15(S)-latanoprost was reported by P. Widomski *et al*. (Widomski et al., 2008), although

previous reports suggested reverse-phase HPLC to be unsuitable for the analysis of the isomers of latanoprost. It is worth noting that the separation of the 5,6-*trans* isomers and 15(*S*)/15(*R*) isomers from the peak of the main substance in the pure substance analysis was achieved in the reverse-phase system for travoprost using the C18 column, and in the case of bimatoprost using the C8 column (Sjöquist et al., 1999; USP, 2021b). Only two analytical methods were reported for the separation of isomers of latanoprost in the drug product. The one method using a chiral mobile phase contains β-cyclodextrin, while the second method uses a chiral stationary phase obtained from cellulose tris-(3,5-dimethylphenylcarbamate) coated on 5 µm Chiralcel OD-RH silica gel (USP-NF, 2021) (Ibrahim et al., 2019). Methods for the determination of the latanoprost content in single as well as in combination products (timolol maleate, netarsudil, brimonidine) are based on the reverse-phase method with stationary phase C18 and a mobile phase consisting of acetonitrile and aqueous phase as acid buffer with pH ~3 in different ratios. All reported methods for latanoprost and travoprost assay related to simple pharmaceutical dosage forms, i.e. eye-drop solutions, while one method was used to determine bimatoprost in Chitosan-Based Ocular Inserts (Franca et al., 2015). The methods for testing the content and purity of bimatoprost seem to be particularly important owing to the absence of compendial methods that could become the basis for the development of methods for various formulations and more complex drug forms. Zezula *et al*. presented an UHPLC-UV method that provides good separation of all potential impurities of bimatoprost, including starting materials, intermediates, isomer degradation products, and at the same time the method can be used to determine the substance content (Zezula et al., 2019). The main issue in the development of new methods is the shortening of the analysis time to as little as three minutes, improving the sensitivity and the environmental and economic aspect related to a reduction in the consumption of toxic and expensive solvents.

Figure 5. *Chemical structures of major process impurities and degradants of latanoprost, bimatoprost, travoprost and tafluprost.*

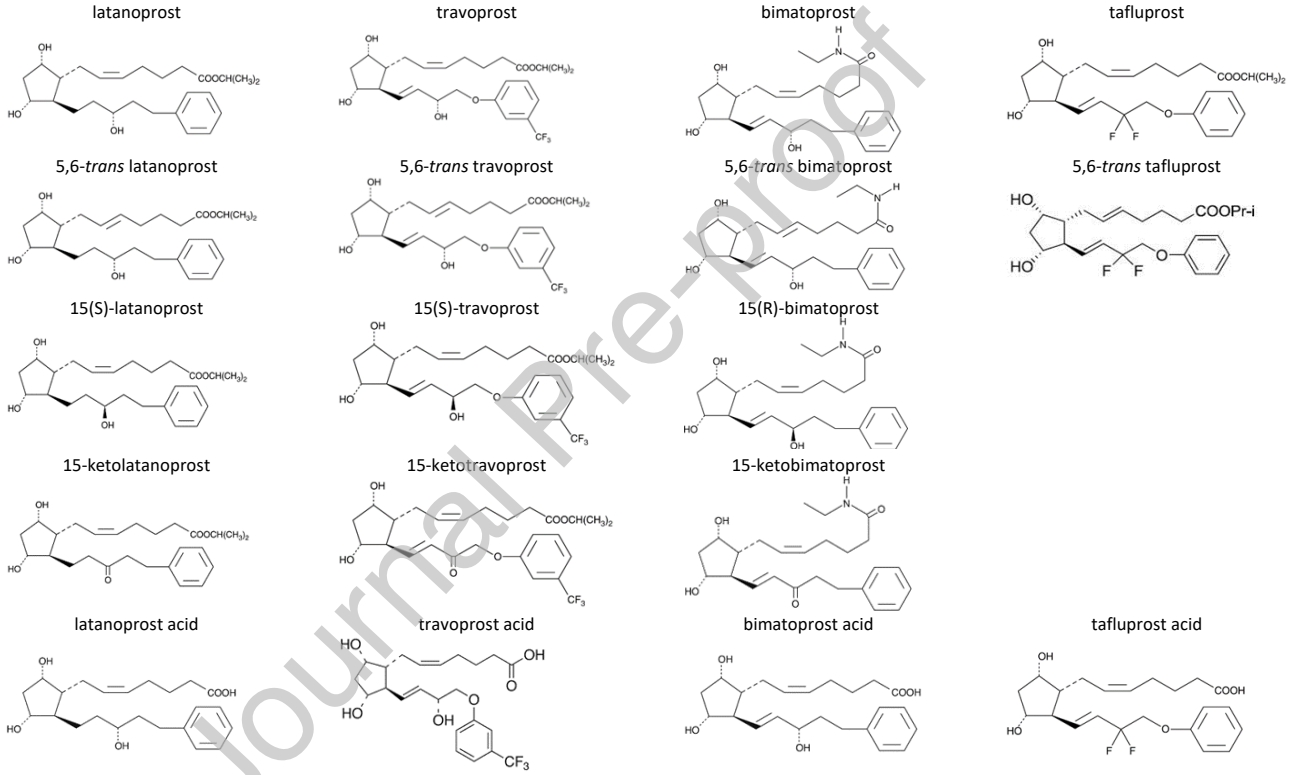


Table 12. *Chromatographic methods for determination of latanoprost, bimatoprost, travoprost and related substances in pure substance and pharmaceutical dosage forms.*

Method name	Chromatographic conditions						Validation results		Reference
	Stationary phase	Mobile phase	Elution mode; Flow rate, Time of analysis	Column temperature	Injection volume	Detector wavelength	LOD – Limit of detection LOQ – Limit of quantification	Linearity, regression equation, correlation coefficient R^2 Range Accuracy: %recovery or bias; Precision: RSD%	
Simultaneous determination of latanoprost and netarsudil in ophthalmic solution	C18 column Agilent 150 mm × 4.6 mm; 5 µm particle size	0.1 N KH_2PO_4 (pH 3.0) + one drop of triethylamine in every 100 ml of KH_2PO_4, acetonitrile (60%:40% v/v)	Isocratic, 1 ml/min, 6 minutes	10°C (30°C)*	10 µl	220 nm	Latanoprost LOD = 0.04 µg/ml LOQ = 0.12 µg/ml	Latanoprost y = 43163x + 510.88; R^2 - 0.999; Range: 0.625-3.75 µg/ml; Accuracy: %recovery - 100.20%; Precision: RSD% (n = 6) - 0.9	(Ramana et al., 2020)
Latanoprost assay in pure substance and pharmaceutical dosage form (eye drops)	C18 column Accurasil endcapped from SGE; 250 mm × 4.6 mm; 5 µm particle size, 93A pore diameter,	Acetonitrile, 0.05 M KH_2PO4 (pH 3.0) (70:30 v/v) pH adjusted by 10% orthophosphoric acid	Isocratic, 1.5 ml/min, 4 minutes	25°C ± 2°C	20 µl	210 nm	NA	y = 72.94x + 0.00043; R^2 - 0.9996; Range: 10.0 - 90 µg/ml; Accuracy: %recovery - 100.40%; RSD% (n = 9) - 1.34 Precision: Repeatability RSD% (n = 6) - 0.9; Intermediate precision (different 3 days) - RSD% (n = 18) - 1.0	(Ashfaq et al., 2006)
Latanoprost assay in pure substance and pharmaceutical dosage form (eye drops)	C18 Agilent Eclipse XDB-C18 column, 150 mm × 4.6 mm; 3.5 µm particle size	Acetonitrile, water (70:30 v/v) containing 0.1% v/v trifluoroacetic acid adjusted to pH 3.0	Isocratic, 1.0 ml/min, 3.3 minutes	25°C	50 µl	205 nm	LOD = 3 ng/ml LOQ = 10 ng/ml	y = 62.24x + 0.26, R^2 - 0.998 Range: 0.0125 - 1 µg/ml; Accuracy: %recovery: 100.15%, RSD% (n = 9) - 1.24; Precision: RSD% (n = 10) - 1.01 (0.1 µg/ml), RSD% (n = 10) - 1.10 (0.5 µg/ml), RSD% (n = 10) - 0.95 (1 µg/ml), Intermediate precision – Inter-day RSD% (n = 10) - 0.97 (0.1 µg/ml), RSD% (n = 10) - 0.91 (0.5 µg/ml), RSD% (n = 10) - 0.89 (1 µg/ml).	(Mansoor and Tas, 2014)

Simultaneous determination of latanoprost, timolol, and benzalkonium chloride in ophthalmic solution	C18 column Inertsil C18, 300 mm × 3.9 mm; 5 µm particle size	Acetonitrile, buffer KH_2PO_4, pH 2.8 (60: 40 v/v); (3.4 g KH_2PO_4 in 1000 ml water and pH adjusted to 2.8 with orthophosphoric acid)	Isocratic, 1.0 ml/min, 14 minutes	30°C	10 µl	Gradient 210 nm latanoprost, benzalkonium chloride and 254 nm timolol	Latanoprost LOD = 0.2 ppm LOQ = 0.6 ppm	Latanoprost Regression equation: NR; R^2 - 0.9994; Range: 2.5 ppm - 15 ppm Accuracy: %recovery - 100.33% (5 ppm); 100.43% (10 ppm); 100.31% (15 ppm); Precision: RSD (n = 6) - 0.34%	(Agarwal et al., 2013)
Simultaneous quantification of latanoprost, timolol and benzalkonium chloride and related substances: latanoprost acid, 15-keto latanoprost and timolol impurity I in ophthalmic formulation, in the presence of degradation products.	Reverse phase cyano column Hypersil BDS CN, 250 mm × 4.6 mm; 5 µm particle size	Mobile phase A: 0.05 M solution of NaH_2PO_4 dehydrate adjusted to pH 3.20 with orthophosphoric acid; Mobile phase B: acetonitrile, methanol (50:50 v/v)	Gradient elution, 1.0 ml/min, 55 minutes	25°C	80 µl	PDA detector λ1 210 nm latanoprost, benzalkonium chloride λ2 295 nm timolol	15-keto latanoprost LOD = 0.022 µg/ml LOQ = 0.068 µg/ml Latanoprost acid LOD = 0.010 µg/ml LOQ = 0.030 µg/ml Latanoprost LOD = 0.006 µg/ml LOQ = 0.018 µg/ml	Latanoprost assay Regression equation: NR; R^2 - 0.9996; Accuracy: %recovery - 99.2% (50%); 101.5% (150%); Precision: RSD% (n = 6) - 0.1 ÷ 0.9; Latanoprost impurities: 15-keto latanoprost R^2 - 0.9950, Accuracy: %recovery - 106.4% (LOQ); 106.0% (150%); Latanoprost acid R^2 - 0.9994; Accuracy: %recovery - 105.5% (LOQ); 101.6% (150%); Latanoprost Accuracy: %recovery - 108.4% (LOQ); 101.4% (150%); Precision: RSD% (n = 6) - 0.5 ÷ 2.0	(Mehta et al., 2010)
Latanoprost assay in ophthalmic solution	packing L1 Sphereclone ODS1, 25 cm × 4.6 mm, 5 µm particle size,	Acetonitrile, solution A, water (125:1:80) Solution A – 17 ml of phosphoric acid diluted to volume 1000 ml with water.	Isocratic 1.0 ml/min RT latanoprost about 8.8 min	NR	50 µl	200 nm	NA	NA	(Monograph, 2012)

Latanoprost organic impurities in ophthalmic solution USP Related compound A and B, imp. 1 (RRT 0.16), imp. 2 (RRT 0.22), *(3S,E)* - Latanoprost isomer	Daicel Chiralcel, OD-R column, 25 cm × 4.6mm, 10 µm particle size	Acetonitrile, water, phosphoric acid (410:590:5)	Isocratic 0.5 ml/min ~ 125 minutes (NLT 2.5 times the RT of latanoprost ~ 42 min)	35°C	50 µl	200 nm	NA	NA	(Monograph, 2012)
Latanoprost assay in ophthalmic formulation containing 0.005% latanoprost	Symmetry C18 column, dimension NR	Water, tetrahydrofuran, methanol (6:1:13 v/v/v) containing 0.05% (w/v) trifluoroacetic acid.	NR	NR	NR	205 nm	NR	Range of testing accuracy 0.45 - 0.55 µg/ml Other validation results NR	(Paolera et al., 2008) 2008
Latanoprost assay and related substances in API Determination of impurities A-G and I, J (chemical name of impurities see Table 9)	Silica gel for chromatography (5 µm), column size 0.25 m × 4.6 mm	Anhydrous ethanol, heptane (6:94 v/v)	Isocratic 1.3 ml/min, About 30 minutes (twice the retention time of latanoprost)	30°C	10 µl	210 nm	NA	NA	(Pharmacopoeia, 2021)
Latanoprost assay and related compound A,B and IDPP in API (chemical name of related compound see Table 9)	chromatography column L3 (USP list); 25 cm × 4.0 mm 5 µm packing	Hexane, dehydrated alcohol (94:6)	Isocratic 1 ml/min	30°C	10 µl	210 nm	NA	NA	(USP-NF, 2021)
Determination of latanoprost impurity H in API (chemical name of impurity H see Table 9)	Base-deactivated end-capped octadecylsilyl silica gel for chromatography, 5 µm, column size 0.15 m × 4 mm	Mobile phase A: phosphoric acid, acetonitrile, water (0.1:30:70 v/v/v), Mobile phase B: phosphoric acid, water, acetonitrile, (0.1:20:80 v/v/v)	Gradient 1 ml/min 15 minutes	60°C	50 µl	200 nm	NA	NA	(Pharmacopoeia, 2021)

Determination of isomers in latanoprost bulk material 5,6-trans latanoprost, 15(S)-latanoprost	Luna NH_2 column 250 mm × 4.6 mm, 5 µm particle size	n-heptane, 2-propanol, acetonitrile (93:6:1 v/v) containing 0.5 ml/L of water	Isocratic 1 ml/min; 50 minutes	25°C	20 µl	210 nm	latanoprost LOD = 0.41 µg/ml LOQ = 1 22 µg/ml 15(S)-latanoprost LOD = 0.39µg/ml LOQ = 1.17µg/ml 5,6 trans latanoprost LOD = 0.42 µg/ml LOQ = 1.29 µg/ml	$R^2 > 0.980$ (latanoprost, 5,6-trans latanoprost, 15(S)-latanoprost) Accuracy: NR Precision: latanoprost RSD% (n = 6, 2 mg/ml) - 0.57; 15(S)-latanoprost RSD% (n = 6, 3 µg/ml) - 1.6; 5,6-trans latanoprost RSD% (n = 6, 60 µg/ml) - 1.2.	(Widomski et al., 2008)
Assay of 16 prostaglandin analogues in cosmetic products latanoprost, latanoprost acid, bimatoprost, bimatoprost acid, bimatoprost isopropyl ester, bimatoprost serinol amide, travoprost, tafluprost, tafluprost ethyl ester, tafluprost ethyl amide and others	Kinetex XB-C18 column 100 mm × 2.1 mm, 2.6 µm coupled to a 0.5 µm KrudKatcher ultra HPLC in line filter 0.004 in i.d.	Mobile phase A: 0.1% formic acid in mixture of water and methanol (95:5 v/v) Phase B 0.1% formic acid in mixture of water and methanol (5:95 v/v)	Gradient 0.50 ml/min 20 minutes	NR	10 µl	MS/MS(ESI+, ESI-)	LOQ 0.25 ng/ml for bimatoprost, tafluprost ethyl amide, 0.5 ng/ml for latanoprost, bimatoprost serinol amide bimatoprost isopropyl ester, travoprost 1 ng/ ml for bimatoprost acid, latanoprost acid, tafluprost, tafluprost ethyl ester	$R^2 > 0.98$ for all tested analogues, Accuracy: %recovery (LOQ level, n=3) bimatoprost 99%; RSD% - 6.11; tafluprost ethyl amide 95%; RSD% - 2.18; latanoprost 106%; RSD% - 1.19; bimatoprost serinol amide 105%; RSD% - 4.14; bimatoprost isopropyl ester 75%; RSD% - 11.8; travoprost 115%; RSD% - 7.31; bimatoprost acid 99%; RSD% - 2.49; latanoprost acid 99%, RSD% - 1.75; tafluprost 109%, RSD% - 4.78; tafluprost ethyl ester - 97%; RSD% - 5.28.	(Wittenberg et al., 2014)
Degradation products of latanoprost in ophthalmic solutions	Purospher STAR(RP-18 endcapped, 3 um) column	Mobile phase A: acetonitrile with 0.1% formic acid Mobile phase B: water with 0.1% formic acid	Linear gradient of 20% acetonitrile to 100% 14 minutes			Detector 1 PDA detector 200 - 700 nm Detector 2 MS(ESI+)	NR	NR	(Velpandian et al., 2015)
Assay of latanoprost in ophthalmic formulations	Purospher STAR(RP-18 endcapped, 3 µm) column	Acetonitrile, water (7:3) containing 0.1 formic acid	Isocratic 0.5 ml/min 5 minutes	22°C	20 µl	MS/MS(ESI+) MRM mode $(M+H)^+$ m/z 433.3→319.2 (transition I) 433.3→379.3 (transition II)	NR	NR	(Velpandian et al., 2015)

Assay of latanoprost, bimatoprost, travoprost in eyelash enhancing cosmetic serum	Kinetex biphenyl 100A 2.1 mm × 100 mm, 2.6 µm	Mobile phase A: 5 mM ammonium acetate with 0.02% formic acid, Mobile phase B: 5 mM ammonium acetate in mixture of acetonitrile and water (95:5 v/v) with 0.02% formic acid	Linear gradient 0.25 ml/min 24 minutes	NR	10 µl	MS/MS (ESI+)	latanoprost LOD 0.3 µg/ml LOQ 1.0 µg/ml bimatoprost LOD 1.5 µg/ml LOQ 5.0 µg/ml travoprost LOD 0.3 µg/ml LOQ 1.0 µg/ml	latanoprost R^2 = 0.991 ± 0.003; %recovery (2 µg/ml) - 102.2%; bimatoprost R^2 = 0.992 ± 0.001 %recovery (2 µg/ml) - 105.0%; travoprost R^2 = 0.990 ± 0.002 %recovery (2 µg/ml) - 94.1%; for each substance range 1-500 µg/ml; precision CV% < 11% (2-450 µg/ml)	(Marchei et al., 2016)
Quantification of latanoprost, brimonidine tartrate and timolol maleate in fixed dose combination eye drops	BDS Hypersil phenyl column; 4.6 mm × 250 mm; 5 µm particle size	Acetonitrile, 25 mM phosphate buffer (KH_2PO_4), pH 4.0, (50:50 v/v)	Isocratic, 1.2 ml/min 6 minutes	NR	20 µl	210 nm	latanoprost LOD = 0.06 µg/ml LOQ = 0.19 µg/ml	latanoprost Y = 3.97 × 10^3 - 966 R^2 = 0.9998 Concentration range 1 - 25 µg/ml Accuracy: %recovery 100.09 ± 1.31% Precision inter-day RSD% - 0.86 (1.0 µg/ml); RSD% - 0.35 (10 µg/ml); RSD% - 0.87 (20 µg/ml).	(Walash and El-Shahen y, 2016)
Latanoprost assay for sorption study	Nucleodur C18 HTec, 125 mm × 4.6 mm, 5 µm particle size	Acetonitrile:water (50:50)	Isocratic, 1.5 ml/min	30°C	NR	210 nm	LOD = 0.01 µg/ml LOQ = 0.05 µg/ml	Concentration range 0.5 - 7 µg/ml; R^2= 0.9990 Y = 38.875x+3548 Accuracy expressed as mean relative bias: 0.5 µg/ml => 8.9%; 3-7 µg/ml => 1.6% Repeatability RSD% (n = 6) - 5.8; Intermediate precision 3 days, RSD% - 6.3	(Le Basle et al., 2017)
Simultaneous determination of latanoprost and timolol in pharmaceutical dosage form. Separations in presence of latanoprost acid, 15(S)-latanoprost	Monolithic column Chromolith Performance RP-18e, 100 mm × 4.6 mm	Mobile phase A: aqueous solution consisting of β-cyclodextrin 11.35 g/l and sodium octane sulfonate 1.5 g/l; Mobile phase B: ethanol	Gradient, 1.2 ml/min	35°C	NR	210 nm	LOD = 0.25 µg/ml LOQ = 1.0 µg/ml	Y = 18.85x+25.19 R^2 = 0.997 Accuracy: % recovery 96.9% (2.5 µg/ml); 102.4% (5.0 µg/ml); 97.7% (25.0 µg/ml); Repeatability RSD% (n = 3) - 0.6 (2.5 µg/ml); RSD% (n = 3) - 0.8 (5.0 µg/ml); RSD% (n = 3) - 0.2 (25.0 µg/ml).	(Ibrahim et al., 2019)

and timolol USP related compound C,E[**].		Initial condition Mobile phase A:B (75:25)							
Determination of bimatoprost in pharmaceutical dosage forms	C18 column Thermo BDS Hypersil C18, 150 mm × 4.6 mm, 5 µm particle size	Phosphate buffer (KH_2PO_4) adjusted to pH 2.8 with orthophosphoric acid, acetonitrile (55:45 v/v)	Isocratic, 1 ml/min 6 minutes	NR	20 µl	210 nm	LOD = 0.137 µg/ml LOQ = 0.416 µg/ml	R^2 = 0.9996; Y = 24008x+45500; Concentration range 50 - 250 µg/ml; Accuracy: %recovery - 99.32%; Repeatability RSD% (n = 5) - 0.62; Intermediate precision (3 days) - RSD% - 0.45.	(Kumar et al., 2011)
Determination of bimatoprost in chitosan-based ocular inserts	C18-column LichroCART 100 250 mm × 4.6 mm, 5 µm particle size	Acetonitrile, methanol, phosphoric acid 0.1% (30:30:40 v/v/v)	Isocratic, 1.0 ml/min	25°C	20 µl	210 nm	LOD = 0.60 µg/ml LOQ = 0.90 µg/ml	R^2 = 0.9994; Y = 26433.5x+880.449; Concentration range 3.00 - 15.0 µg/ml; Accuracy: %recovery - 104,08%; Repeatability RSD% (n = 3) - 3.67 (3.0 µg/ml); RSD% (n = 3) - 0.38 (9.0 µg/ml); RSD% (n = 3) - 1.29 (15.0 µg/ml). Intermediate precision (3 days) RSD% (n = 3) - 2.63 (3.0 µg/ml); RSD% (n = 3) - 0.90 (9.0 µg/ml); RSD% (n = 3) - 0.92 (15.0 µg/ml).	(Franca et al., 2015)

Determination of the chemical purity and assay of bimatoprost. Determination of 9 impurities (starting materials, degradants and isomers)	Acquity BEH C8, 150 mm × 2.1 mm, 1.7 µm particle size	Mobile phase A: 0.01% orthophosphoric acid solution (pH = 3.5), Mobile phase B: acetonitrile 100%	Gradient 0.7 ml/min, 15 minutes	40°C	2 µl	193 nm	8 known impurities and unknown impurities LOD = 0.10 µg/ml; LOQ = 0.15 µg/ml 15-keto bimatoprost LOD = 0.15 µg/ml; LOQ = 0.25 µg/ml.	Bimatoprost content Y = 1.0801x + 4.0184; R^2 = 1.000; Range: 0.40 - 0.60 mg/ml; Accuracy:% recovery 99.8%; Repeatability RSD (n = 6) - 0.12%; Impurities Y = 1.1105x + 0.0002; R^2 = 0.999 acid BT[1] Y = 1.0636x + 0.0011; R^2 = 0.999 5,6-transBT[2] Y = 0.635x + 0.0003; R^2 = 0.999 15-ketoBT[3] Y = 1.2070x + 0.0005; R^2 = 0.999 15-epiBT[4] Accuracy: % recovery, Precision RSD% 98.25%; RSD% - 0.48 (n = 6); acid BT[1]; 99.05%; RSD% - 0.34 (n = 6); 5,6-transBT[2] 99.83%; RSD% - 1.0 (n = 6); 15-ketoBT[3] 99.80%; RSD% - 0.27 (n = 6);15-epiBT[4]	(Zezula et al., 2019)
Simultaneous determination of travoprost and timolol maleate in pharmaceutical formulation	Hypersil BDS C18 250 mm × 4.6 mm; 5 µm particle size	Water (pH 2 adjusted with orthophosphoric acid, methanol (85 :15 v/v)	Isocratic, 0.8 ml/min, 10 minutes (RT travoprost ~5.1 minutes)	40°C	20 µl	233 nm	LOD = 0.002 µg/ml LOQ = 0.007 µg/ml	Travoprost content Concentration range 0.32 - 0.96 µg/ml; R^2 = 0.999; Recovery 98% -101% Repeatability RSD% - 0.61 (n = 6)	(Prasanthi Chengalva, 2016)
Travoprost assay and organic impurities including travoprost related compound A, epoxide derivative, 15-*epi* diastereomer, 5,6-*trans* isomer, 15-keto derivative in travoprost API (chemical name of impurities see Table 10).	Packing L1 5 cm × 4.6 mm,	Acetonitrile, buffer (3:7) Buffer: 2 ml of phosphoric acid to 1 litre of water, adjusted with sodium hydroxide to pH 3.0	Isocratic, 3.0 ml/min	NR	100 µl	220 nm	NA	NA	(USP, 2021a)

Travoprost assay and degradation products including 5,6 -trans travoprost and 15-keto travoprost in travoprost ophthalmic solution (chemical name of impurities see Table 10).	Packing L1 15 cm × 4.6 mm, 5 µm particle size	Acetonitrile:buffer 17:33 2.18 mg/ml sodium 1-octanesulfonate in water adjusted with phosphoric acid to pH 3.5	Isocratic, 2.0 ml/min	NR	100 µl	220 nm	NA	NA	(USP, 2021b)
Travoprost related compound A in travoprost ophthalmic solution (chemical name of related compound A see Table 10).	Packing L1 5 cm × 4.6 mm; 3 µm particle size	Acetonitrile:buffer (6:19) 1 ml of phosphoric acid in water adjusted with phosphoric acid to pH 3.5	Isocratic, 3.0 ml/min	NR	100 µl	220 nm	NR	NR	(USP, 2021b)

* conflicting information regarding temperature of analysis: abstract (30°C), text - material and methods section (10°C);
** - timolol impurity B Ph. Eur, timolol impurity C Ph. Eur.
[1] acid BT – bimatoprost acid, chemical structure, see Figure 5;
[2] 5,6-transBT – 5,6-bimatoprost, chemical structure, see Figure 5;
[3] 15-ketoBT– 15-keto bimatoprost, chemical structure, see Figure 5.;
[4] 15-epiBT– 15-epi bimatoprost, syn. 15(R)-bimatoprost, chemical structure, see Figure 5;
NR: not reported; NA: not applicable (If an analytical method is pharmacopoeial, it is considered as validated, though the results are not included).

6. CONCLUSIONS

The main aim of our paper has been to present the pharmaceutical aspects of medicinal products containing analogues of 2α prostaglandins used in the treatment of glaucoma. On this account, we were particularly interested in drawing attention to the complexity of the technological and analytical issues involved in relation to the physicochemical properties and stability of the substances discussed.

Constant technological improvement in drug formulation is making it possible, for example, to solvate sparingly soluble substances such as prostaglandins, change the pharmaceutical form from a solution to an emulsion, or use modified release systems such as implants. This helps to increase therapy efficacy through improved patient adherence, leading to a reduction in the number of severe cases and, ultimately, better quality of life.

Along with the developments in manufacturing technology, analytical methods are also being improved, making it possible to assess the quality of the designed medicinal products. Advanced techniques allow the detection of substances and their impurities at very low levels, even in the most sophisticated dosage forms.

Impurity profiling of APIs in their finished formulation is one of the challenges facing the pharmaceutical analytical chemist in an industrial environment. The development of suitable analytical procedures for the detection and quantitation of degradation products is crucial for that purpose.

In our publication, we have presented an overview of the chromatographic analytical methods used to determine the content and related substances of latanoprost, travoprost and bimatoprost in substances for pharmaceutical use, medicinal products and cosmetics. Among the 26 methods presented, 12 concern the testing of the content of substances in a simple formulation, 7 in combination drugs and 11 for testing related substances and degradation products. A few methods are used for simultaneous determination of the content and related substances. None of the presented non-compendial methods for determining the substance content in pharmaceutical formulations was selective for 5.6-isomers, which can be present in substance in levels of up to 5% for travoprost and 3.5% for latanoprost. It seems, therefore, that the proposed methods may be biased towards a slight error in cases where isomers are present at a significant but acceptable level owing to the lack of specificity. Taking into account the high permissible limits of impurities with the isomers mentioned above, it seems that the further search for and development of analytical methods enabling the simultaneous determination of the content of active substances, degradation products and related substances that are isomers remains a major challenge. Moreover, the evaluation of all potential degradation products that may arise, depending on the product's composition, is also a vital to ensure the safety and high quality of ophthalmic prostaglandin analogue products used in the treatment of glaucoma.

Author contributions:

Katarzyna Asendrych-Wicik, Jakub Zarczuk, Katarzyna Walaszek, Magdalena Markowicz-Piasecka: Conceptualization, writing - original draft, review and editing. **Magdalena Markowicz-Piasecka, Tomasz Ciach**: Supervision.

Declaration of interest

Katarzyna Asendrych-Wicik and Jakub Zarczuk are employees of Polfa Warszawa S.A. member of Polpharma Group, Katarzyna Walaszek is employee of Polpharma Biologics. The manuscript presents authors view. Authors do not have relevant affiliations or financial involvement with any organization with a financial interest in or financial conflict with the subject matter discussed in the paper.

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