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(54) Title: STABLE POVIDONE-IODINE COMPOSITIONS WITH STEROIDS OR NON-STEROIDAL ANTI-INFLAMMATORIES

(57) Abstract: Disclosed are stable compositions comprising povidone-iodine and a steroid, and methods of making and using such compositions. Also disclosed herein are stable compositions comprising povidone-iodine and an NS AID, and methods of making and using such compositions.

TITLE

Stable Povidone-Iodine Compositions with Steroids or Non-Steroidal Anti-Inflammatories

BACKGROUND

Topical corticosteroids are routinely used to control ocular inflammation. Their mechanism of action involves the inhibition of the immune response and the subsequent tissue destruction that exuberant inflammation may cause. Corticosteroid has the undesirable side effect of limiting the body's intrinsic ability to fight infection. In fact, inopportune steroids usage can worsen the course of an infection secondary to mycobacteria, virus, or fungus. Thus, the use of a combined antimicrobial-steroid medication in ocular infections is recommended only under careful observation of a trained ophthalmologist because of these significant risks. In fact, TOBRADEX (Alcon), the most commonly prescribed combination ophthalmic antimicrobial-steroid drug, specifically lists `viral disease of the cornea and conjunctiva, mycobacteria infection, and fungal infection` as absolute contraindications to its use. Clearly, these combination drugs were not intended to be used in the face of infectious conjunctivitis in which bacterial infection cannot be confirmed.

In U.S. Patent 7,767,217, it is shown that under certain specific conditions, dexamethasone can be combined with povidone-iodine (PVP-I) to form an effective antimicrobial-steroid pharmaceutical composition. However, it is also shown that most preparations which combine PVP-I (or iodine) with a steroid suffer from instability due, in part, to reactivity of the iodine with the steroid. In fact, U.S. Patent 3,886,268 demonstrates the well-known instability of steroid-iodine combinations.

BRIEF SUMMARY

In an embodiment, disclosed herein is an ophthalmic composition suitable for topical administration to an eye, effective for treatment and/or prophylaxis of a microorganism infection or a disorder of at least one tissue of the eye, comprising povidone-iodine in a concentration between 0.01% and 10%, and a steroid selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, hydrocortisone acetate, and combinations thereof.

In an embodiment, the povidone-iodine is between 0.1% and 2.5% by weight. In an embodiment, the povidone-iodine is between 0.5% and 2% by weight. In an embodiment, the total weight of the povidone-iodine and the steroid is between 0.1% and 4.5% in the solution. In an embodiment, the steroid is at a concentration of between 0.01 and 2%. In an embodiment, the steroid is at a concentration of between 0.05 and 1%.

In an embodiment, disclosed herein is a pharmaceutical composition comprising povidone-iodine in a concentration between 0.01% and 10%, and a steroid selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, and combinations thereof, wherein the steroid is at a concentration of between 0.05 and 1%. In an embodiment, the PVP-I is at a concentration of about 0.4%. In an embodiment, the steroid is at a concentration selected from the group consisting of about 0.1%, about 0.05% and about 0.005%.

In an embodiment, an ophthalmic composition further comprises a topical anesthetic which relieves pain. In an embodiment, a topical anesthetic is selected from the group consisting of proparacaine, lidocaine, tetracaine and a combination thereof.

In an embodiment, an ophthalmic composition further comprises a penetration enhancer which enhances the penetration of povidone-iodine into the tissues of the eye. In an embodiment, a penetration enhancer is a topical anesthetic.

In an embodiment, an ophthalmic composition further comprises an antimicrobial preservative. In an embodiment, the antimicrobial preservative is selected from the group consisting of benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, EDTA, sorbic acid, Onamer M and a combination thereof. In an embodiment, the antimicrobial preservative is at a concentration of about 0.001% to 1.0% by weight in said solution.

In an embodiment, an ophthalmic composition further comprises a co-solvent/surfactant. In an embodiment, the co-solvent/surfactant is selected from the group consisting of polysorbate 20, polysorbate 60, polysorbate 80, Pluronic F-68, Pluronic F-84, Pluronic P-103, cyclodextrin, tyloxapol and a combination thereof. In an embodiment, the co-solvent/surfactant is at a concentration of about 0.01% to 2% by weight in said composition.

In an embodiment, an ophthalmic composition further comprises viscosity increasing agent. In an embodiment, the viscosity increasing agent is selected from the group consisting of

polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose, and a combination thereof. In an embodiment, the viscosity increasing agent is at a concentration of about 0.01% to 2% by weight in said solution.

In an embodiment, an ophthalmic composition suitable for topical administration to an eye, effective for treatment and/or prophylaxis of a microorganism infection or a disorder of at least one tissue of the eye, comprises povidone-iodine in a concentration between 0.01% and 10%, and bromfenac. In an embodiment, an ophthalmic composition comprises:

0.3 to 1% (w/w) polyvinylpyrrolidinone-iodine complex;

0.05 to 2% (w/w) bromfenac;

0.005% to 0.02% (w/w) EDTA;

0.01 to 0.5% (w/w) sodium chloride;

0.02 to 0.1% (w/w) tyloxapol;

0.5% to 2% (w/w) sodium sulfate; and

0.1 to 0.5% (w/w) hydroxyethylcellulose.

In an embodiment, an ophthalmic composition is in the form of a solution, suspension, emulsion, ointment, cream, gel, or a controlled-release/sustain-release vehicle.

In an embodiment, a microorganism treated or prevented by prophylaxis using a composition encompassed herein is selected from the group consisting of bacteria, viruses, fungi, and amoebae. In an aspct, bacteria is mycobacteria.

In an embodiment, a disorder treated using an ophthalmic composition encompassed herein is selected from the group consisting of a microorganism infection of at least one tissue of the eye, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis and herpesvirus-related keratitis.

In an embodiment, an ophthalmic composition is used for prophylaxis of infection following corneal abrasion or ocular surgery.

In an embodiment, an ophthalmic composition comprises:

0.3 to 1% (w/w) polyvinylpyrrolidinone-iodine complex;

0.05 to 2% (w/w) steroid;

0.005% to 0.02% (w/w) EDTA;

0.01 to 0.5% (w/w) sodium chloride;

0.02 to 0.1% (w/w) tyloxapol;

0.5% to 2% (w/w) sodium sulfate; and

0.1 to 0.5% (w/w) hydroxyethylcellulose;

wherein the steroid is selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, hydrocortisone acetate, and combinations thereof.

In an embodiment, an ophthalmic composition comprises:

0.4% (w/w) polyvinylpyrrolidinone-iodine complex;

0.1% (w/w) steroid;

0.01% (w/w) EDTA;

0.3% (w/w) sodium chloride salt;

0.05% (w/w) tyloxapol;

0.2% (w/w) sodium sulfate; and

0.25% (w/w) hydroxyethylcellulose;

wherein the steroid is selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, hydrocortisone acetate, and combinations thereof.

In an embodiment, an ophthalmic composition retains 95% of its polyvinylpyrrolidinone-iodine and 95% of its steroid after a period of 1 month. In an embodiment, an ophthalmic composition retains 90% of its polyvinylpyrrolidinone-iodine and 90% of its steroid after a period of 3 months. In an embodiment, an ophthalmic composition retains 90% of its polyvinylpyrrolidinone-iodine and 90% of its steroid after a period of 1 month.

In an embodiment, an ophthalmic composition retains 95% of its polyvinylpyrrolidinone-iodine and 95% of its NSAID after a period of 1 month. In an embodiment, an ophthalmic composition retains 90% of its polyvinylpyrrolidinone-iodine and 90% of its NSAID after a period of 3 months. In an embodiment, an ophthalmic composition retains 90% of its polyvinylpyrrolidinone-iodine and 90% of its NSAID after a period of 1 month.

In an embodiment, an ophthalmic composition comprising polyvinylpyrrolidinone-iodine (PVP-I) and at least one steroid retains about 89% of its PVP-I after a period of 1 month, about 90% of its PVP-I after a period of 1 month, about 91% of its PVP-I after a period of 1 month, about 92% of its PVP-I after a period of 1 month, about 93% of its PVP-I after a period of 1

month, about 94% of its PVP-I after a period of 1 month, about 94% of its PVP-I after a period of 1 month, about 95% of its PVP-I after a period of 1 month, about 96% of its PVP-I after a period of 1 month, about 97% of its PVP-I after a period of 1 month, about 98% of its PVP-I after a period of 1 month, or about 99% of its PVP-I after a period of 1 month.

In an embodiment, an ophthalmic composition comprising polyvinylpyrrolidinone-iodine (PVP-I) and at least one NSAID retains about 89% of its PVP-I after a period of 1 month, about 90% of its PVP-I after a period of 1 month, about 91% of its PVP-I after a period of 1 month, about 92% of its PVP-I after a period of 1 month, about 93% of its PVP-I after a period of 1 month, about 94% of its PVP-I after a period of 1 month, about 94% of its PVP-I after a period of 1 month, about 95% of its PVP-I after a period of 1 month, about 96% of its PVP-I after a period of 1 month, about 97% of its PVP-I after a period of 1 month, about 98% of its PVP-I after a period of 1 month, or about 99% of its PVP-I after a period of 1 month.

In an embodiment, an ophthalmic composition comprising polyvinylpyrrolidinone-iodine (PVP-I) and at least one steroid retains about 89% of its PVP-I after a period of 3 months, about 90% of its PVP-I after a period of 3 months, about 91% of its PVP-I after a period of 3 months, about 92% of its PVP-I after a period of 3 months, about 93% of its PVP-I after a period of 3 months, about 94% of its PVP-I after a period of 3 months, about 94% of its PVP-I after a period of 3 months, about 95% of its PVP-I after a period of 3 months, about 96% of its PVP-I after a period of 3 months, about 97% of its PVP-I after a period of 3 months, about 98% of its PVP-I after a period of 3 months, or about 99% of its PVP-I after a period of 3 months.

In an embodiment, an ophthalmic composition comprising polyvinylpyrrolidinone-iodine (PVP-I) and at least one NSAID retains about 89% of its PVP-I after a period of 3 months, about 90% of its PVP-I after a period of 3 months, about 91% of its PVP-I after a period of 3 months, about 92% of its PVP-I after a period of 3 months, about 93% of its PVP-I after a period of 3 months, about 94% of its PVP-I after a period of 3 months, about 94% of its PVP-I after a period of 3 months, about 95% of its PVP-I after a period of 3 months, about 96% of its PVP-I after a period of 3 months, about 97% of its PVP-I after a period of 3 months, about 98% of its PVP-I after a period of 3 months, or about 99% of its PVP-I after a period of 3 months.

In an embodiment, an ophthalmic composition comprising PVP-I and at least one steroid retains about 89% of its at least one steroid after a period of 1 month, about 90% of its at least

one steroid after a period of 1 month, about 91% of its at least one steroid after a period of 1 month, about 92% of its at least one steroid after a period of 1 month, about 93% of its at least one steroid after a period of 1 month, about 94% of its at least one steroid after a period of 1 month, about 94% of its at least one steroid after a period of 1 month, about 95% of its at least one steroid after a period of 1 month, about 96% of its at least one steroid after a period of 1 month, about 97% of its at least one steroid after a period of 1 month, about 98% of its at least one steroid after a period of 1 month, or about 99% of its at least one steroid after a period of 1 month.

In an embodiment, an ophthalmic composition comprising PVP-I and at least one NSAID retains about 89% of its at least one NSAID after a period of 1 month, about 90% of its at least one NSAID after a period of 1 month, about 91% of its at least one NSAID after a period of 1 month, about 92% of its at least one NSAID after a period of 1 month, about 93% of its at least one NSAID after a period of 1 month, about 94% of its at least one NSAID after a period of 1 month, about 94% of its at least one NSAID after a period of 1 month, about 95% of its at least one NSAID after a period of 1 month, about 96% of its at least one NSAID after a period of 1 month, about 97% of its at least one NSAID after a period of 1 month, about 98% of its at least one NSAID after a period of 1 month, or about 99% of its at least one NSAID after a period of 1 month.

In an embodiment, an ophthalmic composition comprising PVP-I and at least one steroid retains about 89% of its at least one steroid after a period of 3 months, about 90% of its at least one steroid after a period of 3 months, about 91% of its at least one steroid after a period of 3 months, about 92% of its at least one steroid after a period of 3 months, about 93% of its at least one steroid after a period of 3 months, about 94% of its at least one steroid after a period of 3 months, about 94% of its at least one steroid after a period of 3 months, about 95% of its at least one steroid after a period of 3 months, about 96% of its at least one steroid after a period of 3 months, about 97% of its at least one steroid after a period of 3 months, about 98% of its at least one steroid after a period of 3 months, or about 99% of its at least one steroid after a period of 3 months.

In an embodiment, an ophthalmic composition comprising PVP-I and at least one NSAID retains about 89% of its at least one NSAID after a period of 3 months, about 90% of its at least

one NSAID after a period of 3 months, about 91% of its at least one NSAID after a period of 3 months, about 92% of its at least one NSAID after a period of 3 months, about 93% of its at least one NSAID after a period of 3 months, about 94% of its at least one NSAID after a period of 3 months, about 94% of its at least one NSAID after a period of 3 months, about 95% of its at least one NSAID after a period of 3 months, about 96% of its at least one NSAID after a period of 3 months, about 97% of its at least one NSAID after a period of 3 months, about 98% of its at least one NSAID after a period of 3 months, or about 99% of its at least one NSAID after a period of 3 months.

In an embodiment, an ophthalmic composition is an aqueous solution.

In an embodiment, a method is provided for treating and/or prophylaxis of an eye disorder or a microorganism infection of at least one tissue of the eye comprising the step of administering one of more doses of an ophthalmic composition encompassed herein to the eye. In an embodiment, the prophylaxis is prophylaxis of infection following corneal abrasion or ocular surgery. In an embodiment, the eye disorder is selected from the group consisting of a microorganism infection of at least one tissue of the eye, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis and herpesvirus-related keratitis. In an embodiment, the microorganism is a bacteria, virus, fungi, or amoebae. In an embodiment, the bacteria is mycobacteria.

In an embodiment, in a method of treatment, the sum of the povidone-iodine and the steroid is between 0.001 mg to 5 mg per dose. In an embodiment, in a method of treatment, each dose is between 10 microliters to 200 microliters. In an embodiment, in a method of treatment, each dose is between 50 microliters to 80 microliters. In an embodiment, in a method of treatment, the administering step comprises administering a composition encompassed herein to an eye one to four times a day. In an embodiment, in a method of treatment, the administering step comprises administering a composition encompassed herein to an eye one to twenty-four times a day. In an embodiment, in a method of treatment, the method includes storing the composition for at least one month, at least three months, at least six months, or at least 1 year before the administration step.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an image depicting the HPLC-UV/(+)ESI-MS and MS/MS spectral data of dexamethasone phosphate.

Figure 2 is an image depicting the HPLC-UV/(+)ESI-MS and MS/MS spectral data of prednisolone acetate.

Figure 3 is an image depicting the HPLC-UV/(+)ESI-MS and MS/MS spectral data of loteprednol etabonate.

Figure 4 is an image depicting the HPLC-UV/(+)ESI-MS and MS/MS spectral data of difluprednate.

Figure 5 is an image depicting the HPLC/UV chromatograms of PVP-I at the concentration of 200 μg/mL for dexamethasone sodium phosphate.

Figure 6 is an image depicting the HPLC/UV chromatograms of dexamethasone sodium phosphate in PVP-I for Day 0.

Figure 7 is an image depicting the HPLC/UV chromatograms of dexamethasone sodium phosphate.

Figure 8 is an image depicting the HPLC/UV chromatograms of dexamethasone sodium phosphate in PVP-I for two weeks.

Figure 9 is an image depicting the HPLC/UV chromatograms of dexamethasone sodium phosphate in PVP-I for two weeks.

Figure 10 is an image depicting the HPLC/UV chromatograms of dexamethasone sodium phosphate in PVP-I for one month.

Figure 11 is an image depicting the HPLC/UV chromatograms of dexamethasone sodium phosphate in PVP-I for one month.

Figure 12 is an image depicting the HPLC/UV chromatograms (expanded) of dexamethasone sodium phosphate in PVP-I for one month.

Figure 13 is an image depicting the HPLC/UV chromatograms (expanded) of dexamethasone sodium phosphate in PVP-I for one month.

Figure 14 is an image depicting the mass ion chromatograms (MRM Mode) of dexamethasone sodium phosphate in reference standard samples.

Figure 15 is an image depicting the mass ion chromatograms (MRM Mode) of dexamethasone sodium phosphate in one month room temperature stability sample in the presence of PVP-I.

Figure 16 is an image depicting the mass ion chromatograms (MRM Mode) of dexamethasone sodium phosphate in one month 40°C stability sample in the presence of PVP-I.

Figure 17 is an image depicting the HPLC/UV chromatograms of PVP-I at the concentration of 20 μg/mL for prednisolone acetate.

Figure 18 is an image depicting the HPLC/UV chromatograms of prednisolone acetate in PVP-I for Day 0.

Figure 19 is an image depicting the HPLC/UV chromatograms of prednisolone acetate in PVP-I for Day 0.

Figure 20 is an image depicting the HPLC/UV chromatograms of prednisolone acetate in PVP-I for two weeks.

Figure 21 is an image depicting the HPLC/UV chromatograms of prednisolone acetate in PVP-I for two weeks.

Figure 22 is an image depicting the HPLC/UV chromatograms of prednisolone acetate in PVP-I for one month.

Figure 23 is an image depicting the HPLC/UV chromatograms of prednisolone acetate in PVP-I for one month.

Figure 24 is an image depicting the mass ion chromatograms (MRM Mode) of prednisolone acetate in reference standard samples.

Figure 25 is an image depicting the mass ion chromatograms (MRM Mode) of prednisolone acetate in one month room temperature stability sample in the presence of PVP-I.

Figure 26 is an image depicting the mass ion chromatograms (MRM Mode) of prednisolone acetate in one month 40°C stability sample in the presence of PVP-I.

Figure 27 is an image depicting the HPLC/UV chromatograms of PVP-I at the concentration of 40 μg/mL for loteprednol etabonate.

Figure 28 is an image depicting the HPLC/UV chromatograms of loteprednol etabonate in PVP-I for Day 0.

Figure 29 is an image depicting the HPLC/UV chromatograms of loteprednol etabonate in PVP-I for Day 0.

Figure 30 is an image depicting the HPLC/UV chromatograms of loteprednol etabonate in PVP-I for two weeks.

Figure 31 is an image depicting the HPLC/UV chromatograms of loteprednol etabonate in PVP-I for two weeks.

Figure 32 is an image depicting the HPLC/UV chromatograms of loteprednol etabonate in PVP-I for one month.

Figure 33 is an image depicting the HPLC/UV chromatograms of loteprednol etabonate in PVP-I for one month.

Figure 34 is an image depicting the mass ion chromatograms (MRM Mode) of loteprednol etabonate in reference standard samples.

Figure 35 is an image depicting the mass ion chromatograms (MRM Mode) of loteprednol etabonate in one month room temperature stability sample in the presence of PVP-I.

Figure 36 is an image depicting the mass ion chromatograms (MRM Mode) of loteprednol etabonate in one month 40°C stability sample in the presence of PVP-I.

Figure 37 is an image depicting the HPLC/UV chromatograms of PVP-I at the concentration of 400 μg/mL for difluprednate.

Figure 38 is an image depicting the HPLC/UV chromatograms of difluprednate in PVP-I for Day 0.

Figure 39 is an image depicting the HPLC/UV chromatograms of difluprednate in PVP-I for Day 0.

Figure 40 is an image depicting the HPLC/UV chromatograms of difluprednate in PVP-I for two weeks.

Figure 41 is an image depicting the HPLC/UV chromatograms of difluprednate in PVP-I for two weeks.

Figure 42 is an image depicting the HPLC/UV chromatograms of difluprednate in PVP-I for one month.

Figure 43 is an image depicting the HPLC/UV chromatograms of difluprednate in PVP-I for one month.

Figure 44 is an image depicting the mass ion chromatograms (MRM Mode) of difluprednate in reference standard samples.

Figure 45 is an image depicting the mass ion chromatograms (MRM Mode) of difluprednate in one month room temperature stability sample in the presence of PVP-I.

Figure 46 is an image depicting the mass ion chromatograms (MRM Mode) of difluprednate in one month 40°C stability sample in the presence of PVP-I.

DETAILED DESCRIPTION

It is known that iodine, including preparations of PVP-I, reacts chemically with various steroids when combined with a steroid, resulting in an unstable composition, due in part to reactivity of the iodine with the steroid. U.S. Patent 3,886,268 demonstrates the well-known instability of steroid-iodine combinations. It is also known that certain non-steroidal anti-inflammatory compounds ("NSAIDS") also react with iodine. However, U.S. Patent 7,767,217, incorporated herein by reference in its entirety, illustrates that under certain specific conditions, dexamethasone, for example, can be combined with PVP-I to form an effective antimicrobial-steroid pharmaceutical composition. U.S. Provisional Patent Application No. 61/485,475, to which the present application claims priority, is also incorporated herein by reference in its entirety.

Compositions

In an embodiment, compositions disclosed herein comprise PVP-I and a steroid. In an embodiment, compositions disclosed herein comprise PVP-I and an NSAID. In another embodiment, a composition disclosed herein is a pharmaceutical composition. In another embodiment, a composition disclosed herein is an ophthalmic composition.

The invention provides, in part, compositions comprising PVP-I in the range of about 0.01% to about 10% (weight/weight or weight/volume) and a steroid at a concentration of about 0.001% to about 10%. The invention also provides, in part, ophthalmic compositions comprising povidone-iodine in the range of about 0.01% to about 10% (weight/weight or weight/volume) and a therapeutically effective amount of a steroid at a concentration of about 0.001% to about

10%. The invention provides, in part, compositions comprising PVP-I in the range of about 0.01% to about 10% (weight/weight or weight/volume) and an NSAID at a concentration of about 0.001% to about 10%. The invention also provides, in part, ophthalmic compositions comprising povidone-iodine in the range of about 0.01% to about 10% (weight/weight or weight/volume) and a therapeutically effective amount of an NSAID at a concentration of about 0.001% to about 10%.

The affinity of free iodine for reaction with --OH, --SH and --NH functional groups is well described in the literature and forms the basis for the anti-microbial activity of iodine-containing solutions (Rackur H. J. Hosp. Infect., 1985; 6: 13-23, and references therein). Dexamethasone, (9-Fluoro-11.beta., 17, 21-trihydroxy-16.alpha.-methylpregna-1, 4-diene-3, 20-dione) for example, contains three such moieties (--OH) at the 11, 17 and 21 positions. The skilled artisan would conclude that these hydroxyl groups would be prone to covalent substitution reactions by the free iodine generated in the solution equilibrium reaction described above for PVP-I.

In preparing the present compositions, experiments of combinations of various steroids and PVP-I, as well as combinations of various NSAIDS and PVP-I, were performed. It was observed that many formulations were unsuccessful because of the rapid reaction between PVP-I and the added steroid. It was surprising to discover that separate solutions of PVP-I and prednisolone acetate, PVP-I and loteprednol etabonate, PVP-I and hydrocortisone acetate, and PVP-I and difluprednate demonstrate unexpected stability, based on what was previously known in the art. It was also surprising to discover that solutions of PVP-I and bromfenac demonstrate unexpected stability, based on what was previously known in the art. In an embodiment, a combination of PVP-I and one of the steroids or NSAIDS identified above each remains stable for a month or longer.

In an embodiment, a composition comprises PVP-I and prednisolone acetate. In another embodiment, a composition is a pharmaceutical composition comprising PVP-I and prednisolone acetate. In another embodiment, a composition is an ophthalmic preparation comprising PVP-I and prednisolone acetate.

In an embodiment, a composition comprises PVP-I and loteprednol etabonate. In another embodiment, a composition is a pharmaceutical composition comprising PVP-I and loteprednol etabonate. In another embodiment, a composition is an ophthalmic preparation comprising PVP-I and loteprednol etabonate.

In an embodiment, a composition comprises PVP-I and hydrocortisone acetate. In another embodiment, a composition is a pharmaceutical composition comprising PVP-I and hydrocortisone acetate. In another embodiment, a composition is an ophthalmic preparation comprising PVP-I and hydrocortisone acetate.

In an embodiment, a composition comprises PVP-I and difluprednate. In another embodiment, a composition is a pharmaceutical composition comprising PVP-I and difluprednate. In another embodiment, a composition is an ophthalmic preparation comprising PVP-I and difluprednate.

In an embodiment, a composition comprises PVP-I and bromfenac. In another embodiment, a composition is a pharmaceutical composition comprising PVP-I and bromfenac. In another embodiment, a composition is an ophthalmic preparation comprising PVP-I and bromfenac.

Percentages for components of compositions are provided herein as weight/weight (w/w), unless otherwise indicated. For example, 0.6% PVP-I indicates 0.6% PVP-I by weight, with respect to the total weight of 100% for a composition.

In an embodiment, a composition comprises povidone-iodine (PVP-I) at a concentration in the range of about 0.1% to about 2.5%. In another embodiment, a composition comprises povidone-iodine (PVP-I) at a concentration in the range between 0.2 and 1.5%, and in yet another embodiment, between 0.3% and 1.0%. In an embodiment, a composition comprises PVP-I at a concentration in the range of about 0.2 to about 2.0%, about 0.3% to about 1.5%, about 0.36% to about 1.0%, and about 0.4% to about 0.75%. In an embodiment, a composition comprises PVP-I at a concentration of about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9% or about 1.0%. In an

embodiment, a composition comprises povidone-iodine PVP-I at a concentration of 0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.55%, 0.6%, 0.65%, 0.7%, 0.75%, 0.8%, 0.85%, 0.9%, 0.95%, or 1.0%. In another embodiment, a composition comprises PVP-I at a concentration of about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9% or about 10%. In another embodiment, a composition comprises PVP-I at a concentration of about 2% or less, about 3% or less, about 4% or less, about 5% or less, about 6% or less, about 7% or less, about 8% or less, about 9% or less or about 10% or less. In another embodiment, a composition comprises PVP-I at a concentration of about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more or about 10% or more. In another embodiment, a composition comprises PVP-I at a concentration of 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%.

Compositions disclosed herein may comprise one or more steroids. Steroids include, but are not limited to, dexamethasone, dexamethasone alcohol, dexamethasone sodium phosphate, fluromethalone acetate, fluormethalone acetate, fluromethalone alcohol, lotoprednol etabonate, medrysone, prednisolone acetate, prednisolone sodium phosphate, difluprednate, rimexolone, hydrocortisone, hydrocortisone acetate, lodoxamide tromethamine, and any combinations thereof. In an embodiment, a steroid is present in the composition at a level of about 0.001% to about 10%. In an embodiment, a steroid is present in the composition or preparation at a level of 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, or 2.0%. In an embodiment, a steroid is present in the composition or preparation at a level of about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, or about 2.0%. In an embodiment, a steroid is present in the composition or preparation at a level of about 0.001% or less, about 0.002% or less, about 0.003% or less, about 0.004% or less, about 0.005% or less, about 0.006% or less, about 0.007%

or less, about 0.008% or less, about 0.009% or less, about 0.01% or less, about 0.02% or less, about 0.03% or less, about 0.04% or less, about 0.05% or less, about 0.06% or less, about 0.07% or less, about 0.08% or less, about 0.09% or less, about 0.1% or less, about 0.2% or less, about 0.3% or less, about 0.4% or less, about 0.5% or less, about 0.6% or less, about 0.7% or less, about 0.8% or less, about 0.9% or less, about 1.0% or less, about 1.1% or less, about 1.2% or less, about 1.3% or less, about 1.4% or less, about 1.5% or less, about 1.6% or less, about 1.7% or less, about 1.8% or less, about 1.9% or less, or about 2.0% or less. In an embodiment, a steroid is present in the composition or preparation at a level of about 0.001% or more, about 0.002% or more, about 0.003% or more, about 0.004% or more, about 0.005% or more, about 0.006% or more, about 0.007% or more, about 0.008% or more, about 0.009% or more, about 0.01% or more, about 0.02% or more, about 0.03% or more, about 0.04% or more, about 0.05% or more, about 0.06% or more, about 0.07% or more, about 0.08% or more, about 0.09% or more, about 0.1% or more, about 0.2% or more, about 0.3% or more, about 0.4% or more, about 0.5% or more, about 0.6% or more, about 0.7% or more, about 0.8% or more, about 0.9% or more, about 1.0% or more, about 1.1% or more, about 1.2% or more, about 1.3% or more, about 1.4% or more, about 1.5% or more, about 1.6% or more, about 1.7% or more, about 1.8% or more, about 1.9% or more, or about 2.0% or more.

Compositions disclosed herein may comprise one or more NSAIDS. NSAIDS include, but are not limited to, bromfenac, ketorolac, nepafenac, ketotifen fumarate, diclofenac sodium, flurbiprofen sodium, ketorlac tromethamine, suprofen, celecoxib, naproxen, rofecoxib, and any combinations thereof. In an embodiment, an NSAID is present in the composition at a level of about 0.001% to about 10%. In an embodiment, an NSAID is present in the composition or preparation at a level of 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, or 2.0%. In an embodiment, an NSAID is present in the composition or preparation at a level of about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%,

about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, or about 2.0%. In an embodiment, an NSAID is present in the composition or preparation at a level of about 0.001% or less, about 0.002% or less, about 0.003% or less, about 0.004% or less, about 0.005% or less, about 0.006% or less, about 0.007% or less, about 0.008% or less, about 0.009% or less, about 0.01% or less, about 0.02% or less, about 0.03% or less, about 0.04% or less, about 0.05% or less, about 0.06% or less, about 0.07% or less, about 0.08% or less, about 0.09% or less, about 0.1% or less, about 0.2% or less, about 0.3% or less, about 0.4% or less, about 0.5% or less, about 0.6% or less, about 0.7% or less, about 0.8% or less, about 0.9% or less, about 1.0% or less, about 1.1% or less, about 1.2% or less, about 1.3% or less, about 1.4% or less, about 1.5% or less, about 1.6% or less, about 1.7% or less, about 1.8% or less, about 1.9% or less, or about 2.0% or less. In an embodiment, an NSAID is present in the composition or preparation at a level of about 0.001% or more, about 0.002% or more, about 0.003% or more, about 0.004% or more, about 0.005% or more, about 0.006% or more, about 0.007% or more, about 0.008% or more, about 0.009% or more, about 0.01% or more, about 0.02% or more, about 0.03% or more, about 0.04% or more, about 0.05% or more, about 0.06% or more, about 0.07% or more, about 0.08% or more, about 0.09% or more, about 0.1% or more, about 0.2% or more, about 0.3% or more, about 0.4% or more, about 0.5% or more, about 0.6% or more, about 0.7% or more, about 0.8% or more, about 0.9% or more, about 1.0% or more, about 1.1% or more, about 1.2% or more, about 1.3% or more, about 1.4% or more, about 1.5% or more, about 1.6% or more, about 1.7% or more, about 1.8% or more, about 1.9% or more, or about 2.0% or more.

The compositions disclosed herein can be administered as solutions, suspensions, emulsions (dispersions), gels, creams, or ointments in a suitable ophthalmic vehicle. In any of the compositions of this disclosure for topical administration, such as topical administration to the eye, the mixtures are preferably formulated as aqueous solutions at a pH of 3.5 to 6.5. Preferentially the pH is adjusted to between 4 and 5. This pH range may be achieved by the addition of acids/bases to the solution.

In an embodiment, an ophthalmic composition may comprise an optional co-solvent. In another embodiment, the solubility of the components of the present compositions may be enhanced by a surfactant or other appropriate co-solvent in the composition. Such co-solvents or

surfactants include polysorbate -20, -60, and -80, a polyoxyethylene/polyoxypropylene surfactant (e.g. Pluronic F-68, F-84 and P-103), cyclodextrin, tyloxapol, PEG 35 Castor oil (Cremophor EL), polyoxyl 40 Stearate (Myrj 52), other agents known to those skilled in the art, or a combination thereof. Typically, such co-solvents are present at a level of from about 0.01% to about 2% by weight. In an embodiment, a co-solvent is present at a level of about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, or about 2.0%.

In an embodiment, a composition may comprise an optional agent that can increase viscosity. As will be understood by the skilled artisan when armed with the present disclosure, it may be desirable to increase viscosity above that of a simple aqueous solution in order to increase ocular absorption of the active compound, to decrease variability in dispensing the formulation, to decrease physical separation of components of a suspension or emulsion of the formulation and/or to otherwise improve the ophthalmic formulation. Such viscosity-enhancing agents include, but are not limited to, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, other agents known to those skilled in the art, or any combination thereof. Such agents are typically employed at a level of from about 0.01% to about 2% by weight. In an embodiment, such optional agents are present at about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, or about 2.0%.

In another aspect, bioadhesive agents may comprise the compositions, in order to increase the retention time of the drug gradient over a biological substrate. The bioadhesive agents include, but are not limited to, polyvinylpyrrolidone (PVP), xanthan gum, locust bean gum, acacia gum, hydroxypropyl methylcellulose (HPMC), sodium alginate, pectin, gelatin, carbomer, polyvinylalcohol, gellan gum, tragacanth, acacia, and sodium carboxymethyl cellulose, as well as other agents known to those skilled in the art, or any combination thereof.

In yet another embodiment, compositions of the invention may comprise viscoelastic agents such as methyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, polyvinyl alcohol, dextran, chondroitin sulfate and salts thereof, and hyaluronic acid and salts thereof.

In an embodiment, an ophthalmic composition may further comprise one or more of (1) a penetration enhancer which enhances the penetration of povidone-iodine into the tissues of the eye (this may be a topical anesthetic) (2) a co-solvent or a nonionic surface agent - surfactant, which, for example, may be about 0.01% to 2% by weight; (3) a viscosity increasing agent, which, for example, may be about 0.01% to 2% by weight; and (4) a suitable ophthalmic vehicle.

The ophthalmic composition may be in the form of a solution, a suspension, an emulsion, a preparation, an ointment, a cream, a gel, or a controlled-release/sustain-release vehicle. By way of a non-limiting example, the composition may be in the form of a contact lens solution, eyewash, eyedrop, and the like.

Methods

In an embodiment, compositions disclosed herein are useful for preparation of and use as pharmaceutical compositions. In another embodiment, compositions disclosed herein are useful for preparation of and use as compositions other than pharmaceutical compositions.

In an embodiment, compositions disclosed herein are useful for preparation of and use as ophthalmic compositions. In an aspect, a composition of the invention is useful in the treatment of infections of the conjunctiva and cornea. In another aspect, the broad spectrum antimicrobial activity of povidone-iodine enables a composition of the invention to be used to treat ocular conjunctival or corneal infection caused by mycobacteria, viruses, fungi, and amoeba. Additionally the composition is useful in the infectious prophylaxis of patients recovering from ophthalmic surgery.

In an embodiment, an ophthalmic composition is provided that is suitable for topical administration to an eye, effective for treatment and/or prophylaxis of a microorganism infection or a disorder of at least one tissue of the eye. Prophylaxis may be, for example, prophylaxis from infection following surgery, prophylaxis from infection after birth for the newborn, or

prophylaxis from accidental contact with contaminating material. Accidental contact with contaminating material may occur, for example, during surgery or during food processing.

In an aspect, the ophthalmic composition may be used for treatment and/or prophylaxis of a microorganism infection. The microorganism may be a bacterium, a virus, a fungus, or an amoeba, a parasite, or a combination thereof. In an embodiment, the bacteria may be a mycobacterium.

In an aspect, an ophthalmic composition may be used to treat a disorder such as, but not limited to, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis, herpesvirus-related keratitis, ocular surface irregularity, tear deficiency, dry syndrome, meibomian gland dysfunction, blepharitis and uveitis. In another aspect, an ophthalmic composition may be used for prophylaxis of disorders such as conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis, herpesvirus-related keratitis, ocular surface irregularity, tear deficiency, dry syndrome, meibomian gland dysfunction, blepharitis and uveitis.

In another embodiment, the invention is directed to a method for treating and/or prophylaxis of an eye disorder or a microorganism infection of at least one tissue of the eye comprising the step of administering one of more doses of an ophthalmic composition, discussed above, to the eye. The eye disorder may be, for example, a microorganism infection of at least one tissue of the eye, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis, herpes virus-related keratitis, ocular surface irregularity, tear deficiency, dry syndrome, meibomian gland dysfunction, and blepharitis. The microorganism may be bacteria (e.g., mycobacteria), virus, fungi, or amoebae.

In an embodiment, the dose volume administered to a subject may be between about 10 microliters and about 200 microliters, in another embodiment, between about 20 microliters and 100 microliters, and in another embodiment, between about 50 microliters and about 80 microliters, or about one drop per eye. Two or more drops may be added to an eye. Treatment of an eye may be effected by adding a single drop of composition disclosed herein, or by adding two or more drops, as required to achieve the desired result.

In an embodiment, administration frequency may be between 1 and 24 times a day. In an embodiment, administration frequency may be between 1 and 48 times a day. In another

embodiment, administration frequency may be between 2 and 24 times a day. In another embodiment, administration frequency may be between 2 and 4 times a day. In another embodiment, administration frequency may be twice a day. In another embodiment, administration frequency may be once a day. In another embodiment, administration frequency may be less frequent than once a day. In another embodiment, administration frequency may be on demand, as therapeutic treatment is required or desired. In another embodiment, administration frequency may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 48, or 96 times a day.

In an embodiment, a composition disclosed herein is used for prophylaxis and/or treatment of a non-ophthalmic tissue by contacting the tissue with the composition.

The invention is further described by the following examples. It should be recognized that variations based on the inventive features are within the skill of the ordinary artisan, and that the scope of the invention should not be limited by the examples. To properly determine the scope of the present disclosure, an interested party should consider the claims herein, and any equivalent thereof. All patents, patent applications, and references cited herein are hereby incorporated by reference in their entirety.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1: Stability Testing For Steroids Combined With Povidone Iodine

The objective of this study was to determine whether povidone iodine (PVP-I) at the concentration of 4 mg/mL (0.4%) reacts with any of four different steroids (dexamethasone sodium phosphate, prednisolone acetate, loteprednol etabonate, and difluprednate), the active ingredients, in pharmaceutical formulations under both room temperature and 40 °C for a time period of one month.

Dexamethasone sodium phosphate ophthalmic solution (USP, 0.1%) from Alcon Laboratories, prednisolone acetate ophthalmic suspension (USP, 1%) from Alcon Laboratories, loteprednol etabonate ophthalmic suspension (0.5%) from Baush & Lomb, and difluprednate ophthalmic emulsion (0.05%) from Sirion Therapeutics were used for this study. PVP-I was prepared in water at the concentration of 100 mg/mL (10%). One milliliter of the solution, suspension, or emulsion was mixed with 40 μL of 10% PVP-I in 1.5 mL amber glass vials, followed by storage under both room temperature and 40 °C for 2 weeks and one month. The resultant samples in the presence of PVP-I were analyzed using HPLC. The four steroid levels were measured against the reference standard samples stored under room temperature in the absence of PVP-I (0.4%). The One Month stability test samples were analyzed with the reference standard sample using LC-MS/MS Method in MRM mode with three characteristic ion transitions to confirm the identity of four steroids in stability testing samples. The presence of each of the four steroids in the respective pharmaceutical formulations tested was confirmed by LC/UV-MS and MS/MS. Thus, the four pharmaceutical formulations can be used in the study.

After storage under room temperature and 40 °C at the presence of PVP-I (0.4%), the levels of dexamethasone phosphate in two week samples were only 83.04% and 84.57% of those in room temperature and 40 °C Day 0 samples, respectively. The respective data are 84.24% and 84.09% for one month testing, indicating that dexamethasone phosphate was not stable in the presence of PVP-I (0.4%) under the current testing conditions. Three degradation products (D1, D2, and D3) were observed.

After storage under room temperature and 40 °C in the presence of PVP-I (0.4%), the levels of prednisolone acetate in two week testing samples were 99.24% and 96.60% of those in room temperature and 40 °C Day 0 samples, respectively. The respective data are 95.66% and 96.79% for one month testing. Identical mass ion chromatograms and same intensities of mass ion response were observed in the reference standard and one month stability testing samples. The results from both HPLC/UV and LC-MS/MS analysis indicate that prednisolone acetate was stable in the presence of PVP-I (0.4%) under the current testing conditions.

After storage under room temperature and 40 °C in the presence of PVP-I (0.4%), the levels of loteprednol etabonate in two week testing samples were 101.43% and 100.07% of those in room temperature and 40 °C Day 0 samples, respectively. The respective data are 100.72%

and 96.02% for one month testing. Identical mass ion chromatograms and same intensities of mass ion response were observed in the reference standard and one month stability testing samples. The results from both HPLC/UV and LC-MS/MS analysis indicate that loteprednol etabonate was stable in the presence of PVP-I (0.4%) under the current testing conditions.

After storage under room temperature and 40 °C in the presence of PVP-I (0.4%), the levels of difluprednate in two week testing samples were 103.23% and 99.30% of those in room temperature and 40 °C Day 0 samples, respectively. The respective data are 104.47% and 100.24% for one month testing. Identical mass ion chromatograms and same intensities of mass ion response were observed in the reference standard and one month stability testing samples. The results from both HPLC/UV and LC-MS/MS analysis indicate that difluprednate was stable in the presence of PVP-I (0.4%) under the current testing conditions.

1. MATERIALS

1.1 Test Pharmaceutical Formulations

The four steroids and their related pharmaceutical formulations are listed in Table I and Table II.

1.2 Povidone Iodine

Povidone iodine (USP) was obtained from Spectrum Chemicals. Lot No. and expiration date are YQ0429 and January 31, 2011, respectively.

1.3 Solvents, Reagents, and Supplies

OmniSolv® Water was obtained from EM Science. Acetonitrile, methanol, and ammonium acetate were purchased from Sigma-Aldrich.

1.4 Suppliers and Equipment

1.4.1 Supplies

- Serological Pipettes, Kimble Glass Inc
- Wiretrol Micropipettes, Drummond® Scientific Company
- Autosampler Vials, Sun International
- Automatic Pipettes, Gilson

1.4.2 Equipment

- Sartorius Balances, BP301S, Sartorius Corporation

2. METHODS

2.1 Preparation of Stability Test Samples

2.1.1 Preparation of PVP-I Solution (10%, 100mg/mL)

Weigh 1 g of PVP-I and dissolve in 10 mL of water.

2.1.2 Preparation of Stability Test Samples

2.1.2.1 Preparation of Dexamethasone Sodium Phosphate Stability Test Samples

Aliquot 1 mL of ophthalmic solution (USP, 0.1%) into eight amber HPLC vials to give the following samples:

Dexamethasone Sodium Phosphate-1, 2, 3, 4, 5, 6, 7, 8, and 9.

Added 40 µL of PVP-I stock solution (10%) into Dexamethasone Sodium Phosphate-3, 4, 5, and 6, and mixed well to give the following samples:

Dexamethasone Sodium Phosphate+ PVP-I-3, 4, 5, and 6

Store Dexamethasone Sodium Phosphate+PVP-I-3 and 4 on the lab bench at room temperature and store Dexamethasone Sodium Phosphate+PVP-I -5 and 6 in a stability test chamber at 40 °C.

Added 40 µL of water into Dexamethasone Sodium Phosphate-7, 8, and 9, and mixed well to give the following samples:

Dexamethasone Sodium Phosphate+H_2O-7, 8, and 9

Stored Dexamethasone Sodium Phosphate+H_2O-9 on the lab bench at room temperature and store Dexamethasone Sodium Phosphate+H_2O-7 and 8 in a stability test chamber at 40 °C.

Used Dexamethasone Sodium Phosphate+ PVP-I-3 and -5 and Dexamethasone Sodium Phosphate+H_2O-7 for two week stability test. Used Dexamethasone Sodium Phosphate+ PVP-I-4 and -6 and Dexamethasone Sodium Phosphate+H_2O-8 for one month stability test. Used Dexamethasone Sodium Phosphate+H_2O-9 to prepare reference standard.

Stored Dexamethasone Sodium Phosphate-1 and 2 on the lab bench at room temperature. On Week 4, added 40 µL of PVP-I (10%, freshly prepared) and mix well to give Dexamethasone Sodium Phosphate+PVP-I-1 and 2. Used the resultant samples as time zero samples for HPLC analysis.

2.1.2.2 Preparation of Prednisolone Acetate Stability Test Samples

Aliquot ted1 mL of ophthalmic suspension (USP, 1%) into eight amber HPLC vials to give the following samples:

Prednisolone Acetate-1, 2, 3, 4, 5, 6, 7, 8, and 9

Added 40 µL of PVP-I stock solution (10%) into Prednisolone Acetate -3, 4, 5, and 6, and mixed well to give the following samples:

Prednisolone Acetate + PVP-I-3, 4, 5, and 6

Stored Prednisolone Acetate +PVP-I-3 and 4 on the lab bench at room temperature and stored Prednisolone Acetate +PVP-I -5 and 6 in a stability test chamber at 40 °C.

Added 40 µL of water into Prednisolone Acetate -7, 8, and 9, and mixed well to give the following samples:

Prednisolone Acetate +H_2O-7, 8, and 9

Stored Prednisolone Acetate +H_2O-9 on the lab bench at room temperature and stored Prednisolone Acetate +H_2O-7 and 8 in a stability test chamber at 40 °C.

Used Prednisolone Acetate + PVP-I-3 and -5 and Prednisolone Acetate +H_2O-7 for two week stability test. Used Prednisolone Acetate + PVP-I-4 and -6 and Prednisolone Acetate +H_2O-8 for one month stability test. Used Prednisolone Acetate +H_2O-9 to prepare reference standard.

Stored Prednisolone Acetate -1 and 2 on the lab bench at room temperature. On Week 4, added 40 µL of PVP-I (10%, freshly prepared) and mixed well to give Prednisolone Acetate +PVP-I-1 and 2. Used the resultant samples as time zero samples for HPLC analysis.

2.1.2.3 Preparation of Difluprednate Stability Test Samples

Aliquotted 1 mL of Ophthalmic emulsion (0.05%) into eight amber HPLC vials to give the following samples:

Difluprednate-1, 2, 3, 4, 5, 6, 7, 8, and 9

Added 40 µL of PVP-I stock solution (10%) into Difluprednate-3, 4, 5, and 6, and mixed well to give the following samples:

Difluprednate+ PVP-I-3, 4, 5, and 6

Stored Difluprednate+PVP-I-3 and 4 on the lab bench at room temperature and stored Difluprednate+PVP-I -5 and 6 in a stability test chamber at 40 °C.

Added 40 μL of water into Difluprednate-7, 8, and 9, and mixed well to give the following samples:

Difluprednate+H_2O-7, 8, and 9

Stored Difluprednate+H_2O-9 on the lab bench at room temperature and stored Difluprednate+H_2O-7 and 8 in a stability test chamber at 40 °C.

Used Difluprednate+ PVP-I-3 and -5 and Difluprednate+H_2O-7 for two week stability test. Used Difluprednate+ PVP-I-4 and -6 and Difluprednate+H_2O-8 for one month stability test. Used Difluprednate+H_2O-9 to prepare reference standard.

Stored Difluprednate-1 and 2 on the lab bench at room temperature. On Week 4, added 40 μL of PVP-I (10%, freshly prepared) and mix well to give Difluprednate+PVP-I-1 and 2. Used the resultant samples as time zero samples for HPLC analysis.

2.1.2.4 Preparation of Loteprednol Etabonate Stability Test Samples

Aliquotted 1 mL of ophthalmic solution (USP, 0.1%) into eight amber HPLC vials to give the following samples:

Loteprednol Etabonate-1, 2, 3, 4, 5, 6, 7, 8, and 9

Added 40 μL of PVP-I stock solution (10%) into Loteprednol Etabonate-3, 4, 5, and 6, and mix well to give the following samples:

Loteprednol Etabonate+ PVP-I-3, 4, 5, and 6

Stored Loteprednol Etabonate+PVP-I-3 and 4 on the lab bench at room temperature and stored Loteprednol Etabonate+PVP-I -5 and 6 in a stability test chamber at 40 °C.

Added 40 μL of water into Loteprednol Etabonate-7, 8, and 9, and mixed well to give the following samples:

Loteprednol Etabonate+H_2O-7, 8, and 9

Stored Loteprednol Etabonate+H_2O-9 on the lab bench at room temperature and stored Loteprednol Etabonate+H_2O-7 and 8 in a stability test chamber at 40 °C.

Used Loteprednol Etabonate+ PVP-I-3 and -5 and Loteprednol Etabonate+H_2O-7 for two week stability test. Used Loteprednol Etabonate+ PVP-I-4 and -6 and Loteprednol Etabonate+H_2O-8 for one month stability test. Used Loteprednol Etabonate+H_2O-9 to prepare reference standard.

Stored Loteprednol Etabonate-1 and 2 on the lab bench at room temperature. On Week 4, added 40 µL of PVP-I (10%, freshly prepared) and mix well to give Loteprednol Etabonate+PVP-I-1 and 2. Used the resultant samples as time zero samples for HPLC analysis.

2.2 Preparation of Stability Test Samples for HPLC/UV Analysis

2.2.1 Preparation of PVP-I Solution for HPLC/UV Analysis

2.2.1.1 Preparation of PVP-I-4 mg/mL

Mixed 40 µL of PVP-I (10%) with 1 mL of water to give PVP-I-4 mg/mL.

2.2.1.2 Preparation of PVP-I Solution for Dexamethasone Sodium Phosphate Testing

Mixed 100 µL of PVP-I-4 mg/mL with 1.9 mL of water to give PVP-I-200 µg/L for HPLC analysis.

2.2.1.3 Preparation of PVP-I Solution for Prednisolone Acetate Testing

Mixed 100 µL of PVP-I-4 mg/mL with 9.9 mL of acetonitrile:water (1:1) to give PVP-I-40 µg/L.

Mixed 750 µL of PVP-I-40 µg/L with 750 µL of acetonitrile:water (1:1) to give PVP-I-20 µg/L for HPLC analysis.

2.2.1.4 Preparation of PVP-I Solution for Difluprednate Testing

Mixed 100 µL of PVP-I-4 mg/mL with 0.9 mL of methanol to give PVP-I-400 µg/L for HPLC analysis.

2.2.1.5 Preparation of PVP-I Solution for Loteprednol Etabonate Testing

Mixed 100 µL of PVP-I-4 mg/mL with 9.9 mL of acetonitrile:water (1:1) to give PVP-I-40 µg/L for HPLC analysis.

2.2.2 Preparation of Dexamethasone Sodium Phosphate for HPLC/UV Analysis

2.2.2.1 Preparation of Dexamethasone Sodium Phosphate Standard

Mixed 100 µL of Dexamethasone Sodium Phosphate +H_2O-9 with 1.9 mL of H_2O in an HPLC vial to give Dexamethasone Sodium Phosphate+ H_2O-9-50 µg/mL.

2.2.2.2 Preparation of Dexamethasone Sodium Phosphate Stability Test Samples

Mixed 100 µL of Dexamethasone Sodium Phosphate+PVP-1, 2, 3, 4, 5, or 6 with 1.9 mL of H_2O in an HPLC vial to give Dexamethasone Sodium Phosphate+PVP-1, 2, 3, 4, 5, or 6-50µg/mL for HPLC analysis.

2.2.2.3 Preparation of Control Dexamethasone Sodium Phosphate Stability Test Samples

Mixed 100 µL of Dexamethasone Sodium Phosphate+H_2O-7, or 8 with 1.9 mL of H_2O in an HPLC vial to give Dexamethasone Sodium Phosphate+H_2O-7, or 8-50 µg/mL for HPLC analysis.

2.2.3 Preparation of Prednisolone Acetate for HPLC/UV Analysis

2.2.3.1 Preparation of Prednisolone Acetate Standard

Mixed 100 µL of Prednisolone Acetate+H_2O-9 with 9.9 mL of acetonitrile:water (1:1) to give Prednisolone Acetate+H_2O-9-100 µg/mL.

Mixed 750 µL of Prednisolone Acetate+H_2O-9-100 µg/mL with 750 µL of acetonitrile:H2O (1:1) in HPLC vial to give Prednisolone Acetate+H_2O-9-50 µg/mL for HPLC analysis.

2.2.3.2 Preparation of Prednisolone Acetate Stability Test Samples

Mixed 100 µL of Prednisolone Acetate+PVP-I-1, 2, 3, 4, 5, or 6 with 9.9 mL of acetonitrile:water (1:1) to give Prednisolone Acetate+PVP-I-1, 2, 3, 4, 5, or 6-100 µg/mL.

Mixed 750 µL of give Prednisolone Acetate+PVP-I-1, 2, 3, 4, 5, or 6-100 µg/mL with 750 µL of acetonitrile:H2O (1:1) in HPLC vial to give Prednisolone Acetate+PVP-I-1, 2, 3, 4, 5, or 6-50 µg/mL for HPLC analysis.

2.2.3.3 Preparation of Control Prednisolone Acetate Stability Test Samples

Mixed 100 µL of Prednisolone Acetate+H_2O-7, or 8 with 9.9 mL of acetonitrile:water (1:1) to give Prednisolone Acetate+H_2O-7, or 8-100 µg/mL.

Mixed 750 µL of give Prednisolone Acetate+H_2O-7, or 8-100 µg/mL with 750 µL of acetonitrile:H2O (1:1) in HPLC vial to give Prednisolone Acetate+H_2O-7, or 8-50 µg/mL for HPLC analysis.

2.2.4 Preparation of Loteprednol Etabonate for HPLC/UV Analysis

2.2.4.1 Preparation of Loteprednol Etabonate Standard

Mixed 100 µL of Loteprednol Etabonate+H_2O-9 with 9.9 mL of acetonitrile:water (1:1) to give Loteprednol Etabonate+H_2O-9-50 µg/mL.

2.2.4.2 Preparation of Loteprednol Etabonate Stability Test Samples

Mixed 100 µL of Loteprednol Etabonate+PVP-I-1, 2, 3, 4, 5, or 6 with 9.9 mL of acetonitrile:water (1:1) to give Loteprednol Etabonate+PVP-I-1, 2, 3, 4, 5, or 6-50 µg/mL.

2.2.4.3 Preparation of Control Loteprednol Etabonate Stability Test Samples

Mixed 100 µL of Loteprednol Etabonate+H_2O-7, or 8 with 9.9 mL of acetonitrile:water (1:1) to give Loteprednol Etabonate+H_2O-7, or 8-50 µg/mL.

2.2.5 Preparation of Difluprednate for HPLC/UV Analysis

2.2.5.1 Preparation of Difluprednate Standard

Mixed 100 µL of Difluprednate +H_2O-9 with 0.9 mL of methanol in an HPLC vial to give Difluprednate+ H_2O-9-50 µg/mL.

2.2.5.2 Preparation of Difluprednate Stability Test Samples

Mixed 100 µL of Difluprednate+PVP-1, 2, 3, 4, 5, or 6 with 0.9 mL of methanol in an HPLC vial to give Difluprednate+PVP-1, 2, 3, 4, 5, or 6-50µg/mL for HPLC analysis.

2.2.5.3 Preparation of Control Difluprednate Stability Test Samples

Mixed 100 µL of Difluprednate+H_2O-7, or 8 with 0.9 mL of methanol in an HPLC vial to give Difluprednate+H_2O-7, or 8-50 µg/mL for HPLC analysis.

2.3 Preparation of Stability Test Samples for LC-MS/MS Analysis

2.3.1 Preparation of Dexamethasone Sodium Phosphate for LC-MS/MS Analysis

2.3.1.1 Preparation of Dexamethasone Sodium Phosphate Standard

Mixed 100 µL of Dexamethasone Sodium Phosphate+ H_2O-9-50 µg/mL with 0.9 mL of water in an HPLC vial.

2.3.1.2 Preparation of Dexamethasone Sodium Phosphate Stability Test Samples

Mixed 100 µL of Dexamethasone Sodium Phosphate+PVP-4, or 6-50µg/mL with 0.9 mL of water in an HPLC vial.

2.3.2 Preparation of Prednisolone Acetate for HPLC Analysis

2.3.2.1 Preparation of Prednisolone Acetate Standard

Mixed 100 µL of Prednisolone Acetate+H_2O-9-50 µg/mL with 0.9 mL of acetonitrile:water (1:1) in an HPLC vial.

2.3.2.2 Preparation of Prednisolone Acetate Stability Test Samples

Mixed 100 µL of Prednisolone Acetate+PVP-I-4, or 6-50 µg/ with 0.9 mL of acetonitrile:water (1:1) in an HPLC vial.

2.3.3 Preparation of Loteprednol Etabonate for HPLC Analysis

2.3.3.1 Preparation of Loteprednol Etabonate Standard

Mixed 100 µL of Loteprednol Etabonate+H_2O-9-50 µg/mL with 0.9 mL of acetonitrile:water (1:1) in an HPLC vial.

2.3.3.2 Preparation of Loteprednol Etabonate Stability Test Samples

Mixed 100 µL of Loteprednol Etabonate+PVP-I-4, or 6-50 µg/mL with 0.9 mL of acetonitrile:water (1:1) in an HPLC vial.

2.3.4 Preparation of Difluprednate for HPLC Analysis

2.3.4.1 Preparation of Difluprednate Standard

Mixed 100 µL of Difluprednate+ H_2O-9-50 µg/mL with 0.9 mL of methanol in an HPLC vial.

2.3.4.2 Preparation of Difluprednate Stability Test Samples

Mixed 100 µL of Difluprednate+PVP-4, or 6-50µg/mL for HPLC analysis with 0.9 mL of methanol in an HPLC vial.

2.4 HPLC/UV Chromatography

2.4.1 HPLC Method 1 (for Dexamethasone Sodium Phosphate)

HPLC System:	SHIMADZU HPLC system (Pump: LC-10ADVP; Autosampler: SIL-HTC)
UV:	SPD-10AVvp @239 and 210nm
Column:	Waters XTerra MS C18 3.5µm, 2.1x150mm, S/N 019435216117
Column Temperature:	Room Temperature
Autosampler Temperature:	Room Temperature
Injection Vol.:	10 µL
Mobile Phase A:	0.01M NH4OAc in H2O
Mobile Phase B:	ACN
Gradient:	

Time (min)	Flow (mL/min)	A	B
Initial	0.2	100	0
40	0.2	40	60
45	0.2	2	98
50	0.2	2	98
51	0.2	100	100
70	0.2	Stop	

2.4.2 HPLC Method 2 (for Prednisolone Acetate)

The same as Method 1 except the gradient was changed as follows:

Time (min)	Flow (mL/min)	A	B
Initial	0.2	100	0
40	0.2	30	70
45	0.2	2	98
50	0.2	2	98
51	0.2	100	100
70	0.2	Stop	

2.4.3 HPLC Method 3 (for Loteprednol Etabonate and Difluprednate)

The same as Method 1 except the gradient was changed as follows:

Time (min)	Flow (mL/min)	A	B
Initial	0.2	100	0
40	0.2	20	80
45	0.2	2	98
50	0.2	2	98
51	0.2	100	100
70	0.2	Stop	

2.4.4 Date Integration and Calculation

The software provided with the HPLC system (LCSolution™ software, version 1.23, installed by SHIMADZU) was used to integrate the peak area.

The measured peak area was converted into concentrations (μg/mL) using the following equation:

$C_x = A_x \times Cs \div A_x$

where,

C_x = Concentration (μg/mL) of analyte in stability samples

A_x = Peak area from analyte in stability samples

Cs = Concentration (μg/mL) of analyte in standard samples

A_s = Peak area from analyte in standard samples

2.5 Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS)

HPLC Methods: The same as HPLC Method 1,2, and 3 under Section 2.4.

MS Conditions:

Mass Spectrometer:	API 3000 LC/MS/MS System
Ionization Mode:	ESI in Positive mode
ESI:	5,000 V
Temperature:	350 °C
Nebulizer Gas Flow (NEB):	12 psi
Curtain Gas Flow (CUR):	12 units
Turbo-Ion Spray Gas Flow:	7,000-8,000 mL/min
Collision Gas (CAD):	6 units
DP:	30
FP:	80
EP:	8
CXP:	10

Precursor Ion, Product Ion, Collision Energy, and HPLC Retention Time

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (eV)	Retention Time (min)
Dexamethasone Phosphate	473.3	355.2	20	~21.82
	473.3	337.2	20	~21.82
	473.3	237.2	35	~21.82
Prednisolone Acetate	403.1	325.2	20	~27.62
	403.1	307.2	20	~27.62
	403.1	147.1	30	~27.62
Loteprednol Etabonate	467.3	359.2	20	~33.15
	467.3	265.2	30	~33.15
	467.3	147.1	35	~33.15
Difluprednate	509.3	303.2	20	~31.85
	509.3	279.2	20	~31.85
	509.3	101.1	30	~31.85

3. RESULTS

3.1 LC/MS and MS/MS Analyses of Four Formulations

The four formulations used in this study were analyzed by HPLC –UV and MS and MS/MS. The HPLC-UV chromatograms and ESI-MS and MS/MS spectral data were presented in Figure 1 to Figure 4.

The presence of four steroids in the pharmaceutical formulations was confirmed by LC/UV-MS and MS/MS. Thus, the four pharmaceutical formulations can be used for this study.

3.2 HPLC System Suitability Testing

The four standard samples at the concentration of 50 μg/mL were analyzed using HPLC/UV methods developed at PharmaOn. The data are summarized in Table III.

As shown in Table III, the system used in this study was suitable to determine the levels of four steroids in the stability test samples.

3.3 HPLC/UV and LC-MS/MS Analysis of Stability Testing Samples

3.3.1 Dexamethasone Sodium Phosphate

3.3.1.1 PVP-I Sample

PVP-I in solvent at the same concentration as in stability test samples of dexamethasone sodium phosphate was analyzed using HPLC Method 1. The HPLC/UV chromatograms are depicted in Figure 5.

No dexamethasone phosphate was observed in PVP-I sample.

3.3.1.2 Dexamethasone Sodium Phosphate Stability Samples

The Day 0, Two Week, and One Month stability test samples were analyzed with reference standard samples (stored at room temperature in the absence of PVP I) using HPLC Method 1. The sample in the absence of PVP-I with the same concentration of dexamethasone phosphate as those stability samples at the presence of PVP-I was stored in the same stability chamber at 40°C for one month as control sample. The control sample was analyzed under the same conditions. The concentrations of dexamethasone phosphate in the stability samples were calculated. The data were summarized in Table IV. The HPLC/UV chromatograms of all reference standards and stability testing samples are depicted in Figure 6 to Figure 13.

The One Month stability test samples were analyzed with the reference standard sample using LC-MS/MS Method in MRM mode with three characteristic ion transitions to confirm the identity of dexamethasone phosphate in stability testing samples. The mass ion chromatograms are presented in Figure 14 to Figure 16.

Identity of dexamethasone phosphate in reference standard sample and two One month stability test samples was confirmed by LC-MS/MS.

After storage at room temperature and 40°C in the presence of PVP-I (0.4%), the levels of dexamethasone phosphate in two weeks samples were only 83.04% and 84.57% of those in room temperature and 40°C Day 0 samples, respectively (Table IV). The respective data are 84.24% and 84.09% for one month testing (Table IV), indicating that dexamethasone phosphate was not stable in the presence of PVP-I (0.4%) under the current testing conditions.

As shown in Figure 6 to Figure 13, three additional peaks, Degradation Product 1, 2, and 3 (D1, D2, and D2), were observed in both Two Week and/or One Month stability testing samples at the presence of PVP I.

3.3.2 Prednisolone Acetate

3.3.2.1 PVP-I Sample

PVP-I in solvent at the same concentration as in stability test samples of prednisolone acetate was analyzed using HPLC Method 2. The HPLC/UV chromatograms are depicted in Figure 17.

No prednisolone acetate was observed in PVP-I sample.

3.3.2.2 Prednisolone Acetate Stability Samples

The Day 0, Two Week, and One Month stability test samples were analyzed with reference standard samples (stored at room temperature in the absence of PVP I) using HPLC Method 2. The sample in the absence of PVP-I with the same concentration of prednisolone acetate as those stability samples at the presence of PVP-I was stored in the same stability chamber at 40 oC for two week and one month as control samples. The control samples were analyzed under the same conditions. The concentrations of prednisolone acetate in the stability samples were calculated. The data were summarized in Table V. The HPLC/UV chromatograms of all reference standards and stability testing samples are depicted in Figure 18 to Figure 23.

The One Month stability test samples were analyzed with the reference standard sample using LC-MS/MS Method in MRM mode with three characteristic ion transitions to confirm the identity of prednisolone acetate in stability testing samples. The mass ion chromatograms are presented in Figure 24 to Figure 26.

After storage at room temperature and 40°C in the presence of PVP-I (0.4%), the levels of prednisolone acetate in two week testing samples were 99.24% and 96.60% of those in room temperature and 40°C Day 0 samples, respectively (Table V). The respective data are 95.66% and 96.79% for one month testing (Table V). Identical mass ion chromatograms and same intensities of mass ion response were observed in the reference standard and one month stability testing samples. The results from both HPLC/UV and LC-MS/MS analysis indicate that prednisolone acetate was stable in the presence of PVP-I (0.4%) under the current testing conditions.

3.3.3 Loteprednol Etabonate

3.3.3.1 PVP-I Sample

PVP-I in solvent at the same concentration as in stability test samples of loteprednol etabonate was analyzed using HPLC Method 3. The HPLC/UV chromatograms are depicted in Figure 27.

No loteprednol etabonate was observed in PVP-I sample.

3.3.3.2 Loteprednol Etabonate Stability Samples

The Day 0, Two Week, and One Month stability test samples were analyzed with reference standard samples (stored at room temperature in the absence of PVP I) using HPLC Method 3. The sample in the absence of PVP-I with the same concentration of loteprednol etabonate as those stability samples at the presence of PVP-I was stored in the same stability chamber at 40°C for two week and one month as control samples. The control samples were analyzed under the same conditions. The concentrations of loteprednol etabonate in the stability samples were calculated. The data were summarized in Table VI. The HPLC/UV chromatograms of all reference standards and stability testing samples are depicted in Figure 28 to Figure 33.

The One Month stability test samples were analyzed with the reference standard sample using LC-MS/MS Method in MRM mode with three characteristic ion transitions to confirm the identity of loteprednol etabonate in stability testing samples. The mass ion chromatograms are presented in Figure 34 to Figure 36.

After storage at room temperature and 40°C in the presence of PVP-I (0.4%), the levels of loteprednol etabonate in two week testing samples were 101.43% and 100.07% of those in room temperature and 40°C Day 0 samples, respectively (Table VI). The respective data are 100.72% and 96.02% for one month testing (Table VI). Identical mass ion chromatograms and same intensities of mass ion response were observed in the reference standard and one month stability testing samples. The results from both HPLC/UV and LC-MS/MS analysis indicate that loteprednol etabonate was stable in the presence of PVP-I (0.4%) under the current testing conditions.

3.3.4 Difluprednate

3.3.4.1 PVP-I Sample

PVP-I in solvent at the same concentration as in stability test samples of difluprednate was analyzed using HPLC Method 3. The HPLC-UV chromatograms are depicted in Figure 37.

No difluprednate was observed in PVP-I sample.

3.3.4.2 Difluprednate Stability Samples

The Day 0, Two Week, and One Month stability test samples were analyzed with reference standard samples (stored at room temperature in the absence of PVP I) using HPLC Method 3. The sample in the absence of PVP-I with the same concentration of difluprednate as those stability samples at the presence of PVP-I was stored in the same stability chamber at 40°C for two week and one month as control samples. The control samples were analyzed under the same conditions. The concentrations of difluprednate in the stability samples were calculated. The data were summarized in Table VII. The HPLC/UV chromatograms of all reference standards and stability testing samples are depicted in Figure 38 to Figure 43.

The One Month stability test samples were analyzed with the reference standard sample using LC-MS/MS Method in MRM mode with three characteristic ion transitions to confirm the identity of difluprednate in stability testing samples. The mass ion chromatograms are presented in Figure 44 to Figure 46.

After storage at room temperature and 40°C in the presence of PVP-I (0.4%), the levels of difluprednate in two week testing samples were 103.23% and 99.30% of those in room temperature and 40°C Day 0 samples, respectively (Table VII). The respective data are 104.47% and 100.24% for one month testing (Table VII). Identical mass ion chromatograms and same intensities of mass ion response were observed in the reference standard and one month stability testing samples. The results from both HPLC/UV and LC-MS/MS analysis indicate that difluprednate was stable in the presence of PVP-I (0.4%) under the current testing conditions.

TABLES

Table I

Four Pharmaceutical Formulations

Steroids Name	Formulation/Product	Manufacture/Vendor	Lot No.
Dexamethasone Sodium Phosphate	Ophthalmic solution USP, 0.1%	Alcon Laboratories	153643F
Prednisolone Acetate	Ophthalmic Suspension USP, 1%	Alcon Laboratories	148757F
Loteprednol Etabonate	Ophthalmic Suspension, 0.5%	Baush & Lomb	437291
Difluprednate	Ophthalmic emulsion, 0.05%	Sirion Therapeutics	SIR9F001

Table II

Four Steroids

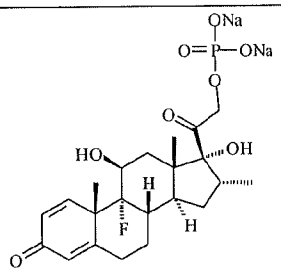
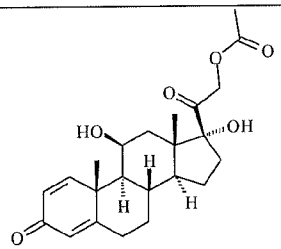
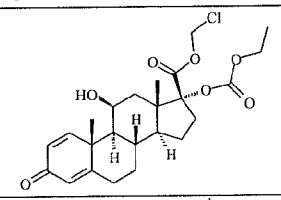
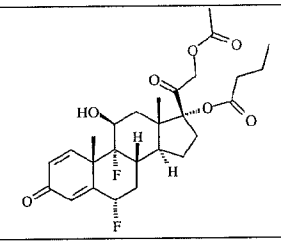
Name	Structure	MW	*Rt* (min)
Dexamethasone Sodium Phosphate		516.41	~21.13
Prednisolone Acetate		402.49	~26.51
Loteprednol Etabonate		466.96	~32.15
Difluprednate		508.56	~31.04

Table III

Summary of System Suitability Testing

Dexamethasone Sodium Phosphate			
Replicate	HPLC Run No.	*Rt* (min)	Peak Area
1	09701005_002	21.16	4860116
2	09701005_003	21.12	4887168
3	09701005_004	21.16	4845056
4	09701005_005	21.12	4841633
5	09701005_006	21.11	4815314
Mean		21.13	4849857
SD		0.024	26369
CV (%)		0.11	0.54
Prednisolone Acetate			
Replicate	HPLC Run No.	*Rt* (min)	Peak Area
1	09701005_012	26.53	5275846
2	09701005_013	26.52	5280425
3	09701005_014	26.54	5197617
4	09701005_015	26.39	5262924
5	09701005_016	26.55	5237854
Mean		26.51	5250933
SD		0.066	34088
CV (%)		0.25	0.65
Loteprednol Etabonate			
Replicate	HPLC Run No.	*Rt* (min)	Peak Area
1	09701005_017	32.19	4352552
2	09701005_018	32.27	4272956
3	09701005_019	32.11	4368753
4	09701005_020	32.11	4281766
5	09701005_021	32.08	4292832
Mean		32.15	4313772
SD		0.078	43748
CV (%)		0.24	1.01
Difluprednate			
Replicate	HPLC Run No.	*Rt* (min)	Peak Area
1	09701005_007	31.02	4746034
2	09701005_008	31.02	4715228
3	09701005_009	31.04	4761819
4	09701005_010	31.06	4715455
5	09701005_011	31.07	4728211
Mean		31.04	4733349
SD		0.023	20288
CV (%)		0.07	0.43

Table IV

Analytical Data Summary of Dexamethasone Sodium Phosphate Stability Testing in PVP-I (0.4 %)

Samples	HPLC Run No.	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (mg/mL) [d]	% of Std	% of Day 0
Day 0									
Std1	09701006_002	20.96	4964292						
Std2	09701006_003	21.23	4873676						
Mean			4918984	50	—	20	1.0000	—	—
Room Temp 1	09701006_004	21.17	5084959		51.69	20	1.0337	103.37	—
Room Temp 2	09701006_005	21.20	5093624		51.78	20	1.0355	103.55	—
Mean			5089292		51.73	20	1.0346	103.46	—
2 Weeks									
Std1	09701004_001	23.91	5019426						
Std2	09701004_002	23.07	5004047						
Mean			5011737	50	—	20	1.0000	—	—
Room Temp	09701004_003	23.08	4305845		42.96	20	0.8592	85.92	83.04
40 °C	09701004_004	23.08	4385137		43.75	20	0.8750	87.50	84.57
One Month									
Std1	09701007_023	20.99	4845855						
Std2	09701007_024	21.03	4810095						
Mean			4827975	50	—	20	1.0000	—	—
Room Temp	09701007_025	21.06	4207982		43.58	20	0.8716	87.16	84.24
40 °C	09701007_026	21.08	4216932		43.67	20	0.8734	87.34	84.42
40 °C	09701007_027	21.07	4184100		43.33	20	0.8666	86.66	83.76
Mean			4200516		43.50	20	0.8700	87.00	84.09
40 °C Control [e]	09701007_028	21.11	4471624		46.31	20	0.9262	92.62	92.62

[a]: Nominal concentration in HPLC samples; [b]: Calculated concentration in HPLC samples; [c]: Dilution factor; [d]: Calculated concentration in stability samples; [e]: Stored at 40 °C without PVP-I.

Table V

Analytical Data Summary of Prednisolone Acetate Stability Testing in PVP-I (0.4 %)

Samples	HPLC Run No.	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (mg/mL) [d]	% of Std	% of Day 0
Day 0									
Std1	09701006_010	26.74	5112497						
Std2	09701006_011	26.75	5081143						
Mean			5096820	50	—	200	10.000	—	—
Room Temp 1	09701006_012	26.76	5342803		52.41	200	10.483	104.83	—
Room Temp 1	09701006_013	26.77	5323574		52.22	200	10.445	104.45	—
Mean			5333189		52.32	20	10.464	104.64	—
2 Weeks									
Std1	09701004_012	27.70	5305927						
Std2	09701004_013	27.73	5317386						
Mean			5311657	50	—	200	10.000	—	—
Room Temp	09701004_014	27.74	5515685		51.92	200	10.384	103.84	99.24
40 °C	09701004_015	27.71	5369264		50.54	200	10.108	101.08	96.60
40 °C Control [e]	09701004_016	27.61	5351149		50.37	200	10.074	100.74	100.74
One Month									
Std1	09701007_012	26.78	5181293						
Std2	09701007_013	26.79	5127543						
Mean			5154418	50	—	200	10.000	—	—
Room Temp	09701007_014	26.81	5159554		50.05	200	10.010	100.10	95.66
40 °C	09701007_015	26.78	5220242		50.64	200	10.128	101.28	96.79
40 °C Control [e]	09701007_016	26.80	5169543		50.15	200	10.029	100.29	100.29

[a]: Nominal concentration in HPLC samples; [b]: Calculated concentration in HPLC samples; [c]: Dilution factor; [d]: Calculated concentration in stability samples; [e]: Stored at 40 °C without PVP-I.

Table VI

Analytical Data Summary of Loteprednol Etabonate Testing in PVP-I (0.4 %)

Samples	HPLC Run No.	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (mg/mL) [d]	% of Std	% of Day 0
Day 0									
Std1	09701006_014	32.41	4172610						
Std2	09701006_015	32.41	4193226						
Mean			4182918	50	—	100	5.0000	—	—
Room Temp 1	09701006_016	32.45	4224688		50.50	100	5.0499	101.00	—
Room Temp 2	09701006_017	32.27	4180845		49.98	100	4.9975	99.95	—
Mean	09701006_017	32.27	4202767		50.24	20	5.0237	100.48	—
2 Weeks									
Std1	09701004_017	32.87	4460467						
Std2	09701004_018	33.02	4431159						
Mean			4445813	50	—	100	5.0000	—	—
Room Temp	09701004_019	33.03	4530572		50.95	100	5.0953	101.91	101.43
40 °C	09701004_020	32.99	4470012		50.27	100	5.0272	100.54	100.07
40 °C Control [e]	09701004_021	32.98	4521010		50.85	100	5.0846	101.69	101.69
One Month									
Std1	09701007_017	32.45	4074874						
Std2	09701007_018	32.30	4068504						
Mean			4071689	50	—	100	5.0000	—	—
Room Temp	09701007_019	32.34	4120353		50.60	100	5.0598	101.20	100.72
40 °C	09701007_020	32.48	3928248		48.24	100	4.8239	96.48	96.02
40 °C Control [e]	09701007_021	32.46	3975565		48.82	100	4.8820	97.64	97.64

[a]: Nominal concentration in HPLC samples; [b]: Calculated concentration in HPLC samples; [c]: Dilution factor; [d]: Calculated concentration in stability samples; [e]: Stored at 40 °C without PVP-I.

Table VII

Analytical Data Summary of Difluprednate Stability Testing in PVP-I (0.4 %)

Samples	HPLC Run No.	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (mg/mL) [d]	% of Std	% of Day 0
Day 0									
Std1	09701006_006	31.17	4647615						
Std2	09701006_007	31.10	4757011						
Mean			4702313	50	—	10	0.5000	—	—
Room Temp 1	09701006_008	31.17	4503933		47.89	10	0.4789	95.78	—
Room Temp 2	09701006_009	31.16	4548076		48.36	10	0.4836	96.72	—
Mean	09701006_009	31.16	4526005		48.13	20	0.4813	96.25	—
2 Weeks									
Std1	09701004_007	31.76	4849758						
Std2	09701004_008	31.76	4871971						
Mean			4860865	50	—	10	0.5000	—	—
Room Temp	09701004_009	31.75	4829559		49.68	10	0.4968	99.36	103.23
40 °C	09701004_010	31.74	4645691		47.79	10	0.4779	95.57	99.30
40 °C Control [e]	09701004_011	31.85	4350242		44.75	10	0.4475	89.50	89.50
One Month									
Std1	09701007_007	31.26	4519656						
Std2	09701007_008	31.21	4538123						
Mean			4528890	50	—	10	0.5000	—	—
Room Temp	09701007_009	31.20	4554140		50.28	10	0.5028	100.56	104.47
40 °C	09701007_010	31.21	4369678		48.24	10	0.4824	96.48	100.24
40 °C Control [e]	09701007_011	31.24	4432171		48.93	10	0.4893	97.86	97.86

[a]: Nominal concentration in HPLC samples; [b]: Calculated concentration in HPLC samples; [c]: Dilution factor; [d]: Calculated concentration in stability samples; [e]: Stored at 40 °C without PVP-I.

Example 2: Stability Testing For Steroids and NSAIDS Combined With 0.6% Povidone Iodine

Steroids and NSAIDS were mixed with PVP-I at the concentration of 0.6% w/w on Day 1. The resultant mixtures will be split to glass vials and stored at room temperature. fluorometholone alcohol, medrysone, prednisone sodium phosphate, rimexolone, hydrocortisone, hydrocortisone acetate, lodoxamide tromethamine, nepafenac, bromfenac, and ketorolac. Testing timepoints included day 0 (Time Zero), and week 4. Tests were conducted at room temperature. The testing samples were analyzed using liquid chromatography and tandem mass spectrometry (LC/MS/MS) methods at Day 0, and Week 4. The steroids and NSAIDS standards were also analyzed and steroids and NSAIDS levels in testing samples were determined.

Rimexolone, hydrocortisone acetate, lodoxamide, and bromfenac samples appeared to be stable. Nepafenac was generally stable, but to a lesser degree. Prednisone sodium phosphate was stable to a lesser degree than nepafenac. In an embodiment, a result wherein about 10% or greater reduction in concentration of a compound of interest is observed is an indication that the compound is not stable. In an embodiment, a result wherein a reduction in the concentration of a compound of interest is observed, but about less than 10% reduction in concentration of a compound of interest is observed, is an indication that the compound is semi-stable. In an embodiment, a result wherein there is substantially no reduction in concentration of a compound of interest observed is an indication that the compound is stable.

Table VIII illustrates the analytical data summary of bromfenac stability testing in 0.6% PVP-I at room temperature. Table IX illustrates the analytical data summary of hydrocortisone acetate stability testing in 0.6% PVP-I at room temperature. Table X illustrates the analytical data summary of rimexolone stability testing in 0.6% PVP-I at room temperature. Table XI illustrates the analytical data summary of prednisone sodium phosphate stability testing in 0.6% PVP-I at room temperature. Table XII illustrates the analytical data summary of nepafenac stability testing in 0.6% PVP-I at room temperature. Table XIII illustrates the analytical data summary of fluorometholone stability testing in 0.6% PVP-I at room temperature. For Tables VIII-XIII, a: Nominal concentration in HPLC samples; b: Calculated concentration in HPLC samples; c: Dilution factor; d: Calculated concentration in stability samples; e: Spiked 50μL of H_2O and stored at room temperature without PVP-I.

Table VIII: Bromfenac testing.

Samples	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (μg/mL) [d]	% of Std	% of Day 0
Standard 1	24.925	11390037	90	—	10	900	—	—
Standard 2	25.034	11288449	90	—	10	900	—	—
Mean	24.980	11339243	90	—	10	900	—	—
Day 0								
Replicate 1	24.900	11310534	90	89.77	10	897.7	99.74	—
Replicate 2	24.889	11107933	90	88.16	10	881.6	97.96	—
Mean	24.895	11209234	90	88.97	10	889.7	98.86	—
Four Weeks								
Replicate 1	24.960	11211003	90	88.98	10	889.8	98.87	100.01
Replicate 2	24.963	11066657	90	87.84	10	878.4	97.6	98.73
Mean	24.962	11138830	90	88.41	10	884.1	98.23	99.37
Control [e]	24.978	11342445	90	90.03	10	900.3	100.03	101.19

Table IX: Hydrocortisone acetate testing.

Samples	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (μg/mL) [d]	% of Std	% of Day 0
Standard 1	29.087	9578995	100	—	50	5000	—	—
Standard 2	29.215	9456921	100	—	50	5000	—	—
Mean	29.151	9517958	100	—	50	5000	—	—
Day 0								
Replicate 1	29.067	9672596	100	101.62	50	5081	101.62	—
Replicate 2	29.107	9472035	100	99.52	50	4976	99.52	—
Mean	29.087	9572316	100	100.57	50	5029	100.57	—
Four Weeks								
Replicate 1	29.125	9627042	100	101.15	50	5058	101.15	100.58
Replicate 2	29.127	9699896	100	101.91	50	5096	101.91	101.33
Mean	29.126	9663469	100	101.53	50	5077	101.53	100.95
Control [e]	29.178	9676282	100	101.66	50	5083	101.66	101.08

Table X: Rimexolone testing.

Samples	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (μg/mL) [d]	% of Std	% of Day 0
Standard 1	39.98	3399891	100	—	100	10,000	—	—
Standard 2	39.961	3404392	100	—	100	10,000	—	—
Mean	39.971	3402142	100	—	100	10,000	—	—
Day 0								
Replicate 1	40.004	3362494	100	98.83	100	9883	98.83	—
Replicate 2	40.018	3418997	100	100.5	100	10050	100.5	—
Mean	40.011	3390746	100	99.67	100	9967	99.67	—
Four Weeks								
Replicate 1	40.035	3398853	100	99.9	100	9990	99.9	100.23
Replicate 2	39.948	3375059	100	99.2	100	9920	99.2	99.53
Mean	39.992	3386956	100	99.55	100	9955	99.55	99.88
Control [e]	20.117	3303121	100	97.09	100	9709	97.09	97.41

Table XI: Prednisone sodium phosphate testing.

Samples	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (μg/mL) [d]	% of Std	% of Day 0
Standard 1	26.61	8422981	100	—	50	5000	—	—
Standard 2	26.748	8470831	100	—	50	5000	—	—
Mean	26.679	8446906	100	—	50	5000	—	—
Day 0								
Replicate 1	26.843	8272276	100	97.93	50	4897	97.93	—
Replicate 2	26.717	8243394	100	97.59	50	4880	97.59	—
Mean	26.780	8257835	100	97.76	50	4888	97.76	—
Four Weeks								
Replicate 1	26.608	7853275	100	92.97	50	4649	92.97	95.1
Replicate 2	26.738	7946048	100	94.07	50	4704	94.07	96.23
Mean	26.673	7899661.5	100	93.52	50	4676	93.52	95.66
Control [e]	26.477	8495335	100	100.57	50	5029	100.57	102.87

Table XII: Nepafenac testing (270nm).

Samples	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (μg/mL) [d]	% of Std	% of Day 0
Standard 1	34.589	727	50	—	100	5,000	—	—
Standard 2	34.580	729	50	—	100	5,000	—	—
Mean	34.585	728	50	—	100	5,000	—	—
Day 0								
Replicate 1	34.568	715	50	49.11	100	4911	98.22	—
Replicate 2	34.548	722	50	49.59	100	4959	99.18	—
Mean	34.558	719	50	49.35	100	4935	98.7	—
Four Weeks								
Replicate 1	34.538	703	50	48.28	100	4828	96.56	97.83
Replicate 2	34.577	694	50	47.66	100	4766	95.32	96.58
Mean	34.558	698.5	50	47.97	100	4797	95.94	97.2
Control [e]	34.570	719	50	49.38	100	4938	98.76	100.06

Table XIII: Fluorometholone testing.

Samples	*Rt* (min)	Peak Area	Nominal Conc. (μg/mL) [a]	Calc Conc. (μg/mL) [b]	DF [c]	Calc Conc. (μg/mL) [d]	% of Std	% of Day 0
Standard 1	38.664	1872	50	—	20	1,000	—	—
Standard 2	38.614	1877	50	—	20	1,000	—	—
Mean	38.639	1875	50	—	20	1,000	—	—
Day 0								
Replicate 1	38.648	1901	50	50.71	20	1014	101.42	—
Replicate 2	38.646	1896	50	50.57	20	1011	101.14	—
Mean	38.647	1899	50	50.64	20	1013	101.28	—
Four Weeks								
Replicate 1	38.611	1861	50	49.64	20	993	99.28	98.03
Replicate 2	38.613	1877	50	50.07	20	1001	100.14	98.87
Mean	38.612	1869	50	49.85	20	997	99.7	98.44
Control [e]	38.602	1860	50	49.61	20	992	99.22	97.97

It is to be understood that at least some of the descriptions of the invention have been simplified to focus on elements that are relevant for a clear understanding of the invention, while eliminating, for purposes of clarity, other elements that those of ordinary skill in the art will appreciate may also comprise a portion of the invention. However, because such elements are well known in the art, and because they do not necessarily facilitate a better understanding of the invention, a description of such elements is not provided herein.

Further, to the extent that the method does not rely on the particular order of steps set forth herein, the particular order of the steps should not be construed as limitation on the claims. The claims directed to the method of the present invention should not be limited to the performance of their steps in the order written, and one skilled in the art can readily appreciate that the steps may be varied and still remain within the spirit and scope of the present invention.

CLAIMS

1. An ophthalmic composition suitable for topical administration to an eye, effective for treatment and/or prophylaxis of a microorganism infection or a disorder of at least one tissue of the eye, comprising

a) povidone-iodine in a concentration between 0.01% and 10%, and

b) a steroid selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, hydrocortisone acetate, and combinations thereof.

2. The ophthalmic composition of claim 1 wherein said povidone-iodine is between 0.1% and 2.5% by weight.

3. The ophthalmic composition of claim 1 wherein said povidone-iodine is between 0.5% and 2% by weight.

4. The ophthalmic composition of claim 1 wherein a total weight of said povidone-iodine and said steroid is between 0.1% and 4.5% in said solution.

5. The ophthalmic composition of claim 1 wherein said steroid is at a concentration of between 0.01 and 2%.

6. The ophthalmic composition of claim 1 wherein said steroid is at a concentration of between 0.05 and 1%.

7. A pharmaceutical composition comprising:

a) povidone-iodine in a concentration between 0.01% and 10%, and

b) a steroid selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, and combinations thereof;

wherein said steroid is at a concentration of between 0.05 and 1%.

8. The composition of claim 7, wherein the PVP-I is at a concentration of about 0.4%.

9. The composition of claim 7, wherein the steroid is at a concentration selected from the group consisting of about 0.1%, about 0.05% and about 0.005%.

10. The ophthalmic composition of claim 1 wherein said composition further comprises a topical anesthetic which relieves pain.

11. The ophthalmic composition of claim 10 wherein said topical anesthetic is selected from the group consisting of proparacaine, lidocaine, tetracaine and a combination thereof.

12. The ophthalmic composition of claim 1 wherein said composition further comprises a penetration enhancer which enhances the penetration of povidone-iodine into the tissues of the eye.

13. The ophthalmic composition of claim 12 wherein said penetration enhancer is a topical anesthetic.

14. The ophthalmic composition of claim 1 wherein said composition further comprises an antimicrobial preservative.

15. The ophthalmic composition of claim 14 wherein said antimicrobial preservative is selected from the group consisting of benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, EDTA, sorbic acid, Onamer M and a combination thereof.

16. The ophthalmic composition of claim 14 wherein said antimicrobial preservative is at a concentration of about 0.001% to 1.0% by weight in said solution.

17. The ophthalmic composition of claim 1 wherein said composition further comprises a co-solvent/surfactant.

18. The ophthalmic composition of claim 17 wherein said co-solvent/surfactant is selected from the group consisting of polysorbate 20, polysorbate 60, polysorbate 80, Pluronic F-68, Pluronic F-84, Pluronic P-103, cyclodextrin, tyloxapol and a combination thereof.

19. The ophthalmic composition of claim 17 wherein said co-solvent/surfactant is at a concentration of about 0.01% to 2% by weight in said composition.

20. The ophthalmic composition of claim 1 wherein said composition further comprises viscosity increasing agent.

21. The ophthalmic composition of claim 20 wherein said viscosity increasing agent is selected from the group consisting of polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose, and a combination thereof.

22. The ophthalmic composition of claim 20 wherein said viscosity increasing agent is at a concentration of about 0.01% to 2% by weight in said solution.

23. The ophthalmic composition of claim 1, wherein said composition is in the form of a solution, suspension, emulsion, ointment, cream, gel, or a controlled-release/sustain-release vehicle.

24. The ophthalmic composition of claim 1, wherein said microorganism is selected from the group consisting of bacteria, viruses, fungi, and amoebae.

25. The ophthalmic composition of claim 24 wherein said bacteria is mycobacteria.

26. The ophthalmic composition of claim 1 wherein said eye disorder is selected from the group consisting of a microorganism infection of at least one tissue of the eye, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis and herpesvirus-related keratitis.

27. The ophthalmic composition of claim 1 wherein said prophylaxis is prophylaxis of infection following corneal abrasion or ocular surgery.

28. The ophthalmic composition of claim 1, comprising:
0.3 to 1% (w/w) polyvinylpyrrolidinone-iodine complex;
0.05 to 2% (w/w) steroid;
0.005% to 0.02% (w/w) EDTA;
0.01 to 0.5% (w/w) sodium chloride;
0.02 to 0.1% (w/w) tyloxapol;
0.5% to 2% (w/w) sodium sulfate; and
0.1 to 0.5% (w/w) hydroxyethylcellulose;

wherein said steroid is selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, hydrocortisone acetate, and combinations thereof.

29. The ophthalmic composition of claim 1, comprising:
0.4% (w/w) polyvinylpyrrolidinone-iodine complex;
0.1% (w/w) steroid;
0.01% (w/w) EDTA;
0.3% (w/w) sodium chloride salt;
0.05% (w/w) tyloxapol;

0.2% (w/w) sodium sulfate; and
0.25% (w/w) hydroxyethylcellulose;

wherein said steroid is selected from the group consisting of prednisolone acetate, loteprednol etabonate, difluprednate, hydrocortisone acetate, and combinations thereof.

30. The ophthalmic composition of claim 1 wherein said composition retains 95% of its polyvinylpyrrolidinone-iodine and 95% of its steroid after a period of 1 month.

31. The ophthalmic composition of claim 1 wherein said composition retains 90% of its polyvinylpyrrolidinone-iodine and 90% of its steroid after a period of 3 months.

32. The ophthalmic composition of claim 1 wherein said composition retains 90% of its polyvinylpyrrolidinone-iodine and 90% of its steroid after a period of 1 month.

33. The ophthalmic composition of claim 1 wherein said composition is an aqueous solution.

34. A method for treating and/or prophylaxis of an eye disorder or a microorganism infection of at least one tissue of the eye comprising the step of administering one of more doses of an ophthalmic composition of claim 1 to said eye.

35. The method of claim 34 wherein said prophylaxis is prophylaxis of infection following corneal abrasion or ocular surgery.

36. The method of claim 34 wherein said eye disorder is selected from the group consisting of a microorganism infection of at least one tissue of the eye, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, epithelial keratitis, stromal keratitis and herpesvirus-related keratitis.

37. The method of claim 34, wherein said microorganism is a bacteria, virus, fungi, or amoebae.

38. The method of claim 37 wherein said bacteria is mycobacteria.

39. The method of claim 34 wherein the sum of said povidone-iodine and said steroid is between 0.001 mg to 5 mg per dose.

40. The method of claim 34 wherein each dose is between 10 microliters to 200 microliters.

41. The method of claim 34 wherein each dose is between 50 microliters to 80 microliters.

42. The method of claim 34 wherein said administering comprises administering said solution to said eye one to four times a day.

43. The method of claim 34 wherein said administering comprises administering said solution to said eye one to twenty-four times a day.

44. The method of claim 34 further comprising the step of storing the composition for at least one month, at least three months, at least six months, or at least 1 year before said administration step.

45. An ophthalmic composition suitable for topical administration to an eye, effective for treatment and/or prophylaxis of a microorganism infection or a disorder of at least one tissue of the eye, comprising

a) povidone-iodine in a concentration between 0.01% and 10%, and

b) bromfenac.

46. The ophthalmic composition of claim 45, comprising:

0.3 to 1% (w/w) polyvinylpyrrolidinone-iodine complex;

0.05 to 2% (w/w) bromfenac;

0.005% to 0.02% (w/w) EDTA;

0.01 to 0.5% (w/w) sodium chloride;

0.02 to 0.1% (w/w) tyloxapol;

0.5% to 2% (w/w) sodium sulfate; and

0.1 to 0.5% (w/w) hydroxyethylcellulose.

HPLC-UV/(+)ESI-MS and MS/MS Spectral Data of Dexamethasone Phosphate

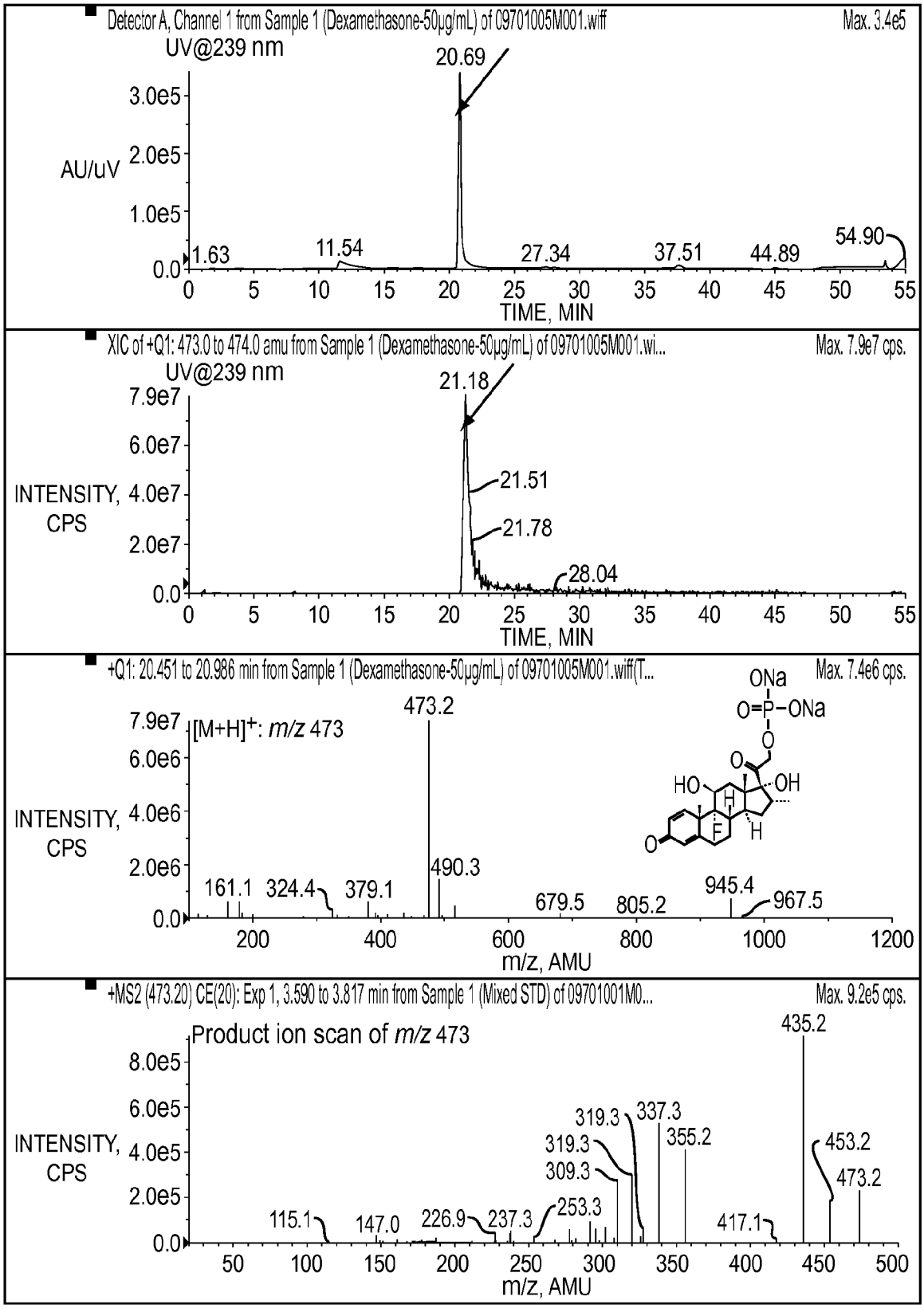


FIG. 1

HPLC-UV/(+)ESI-MS and MS/MS Spectral Data of Prednisolone Acetate

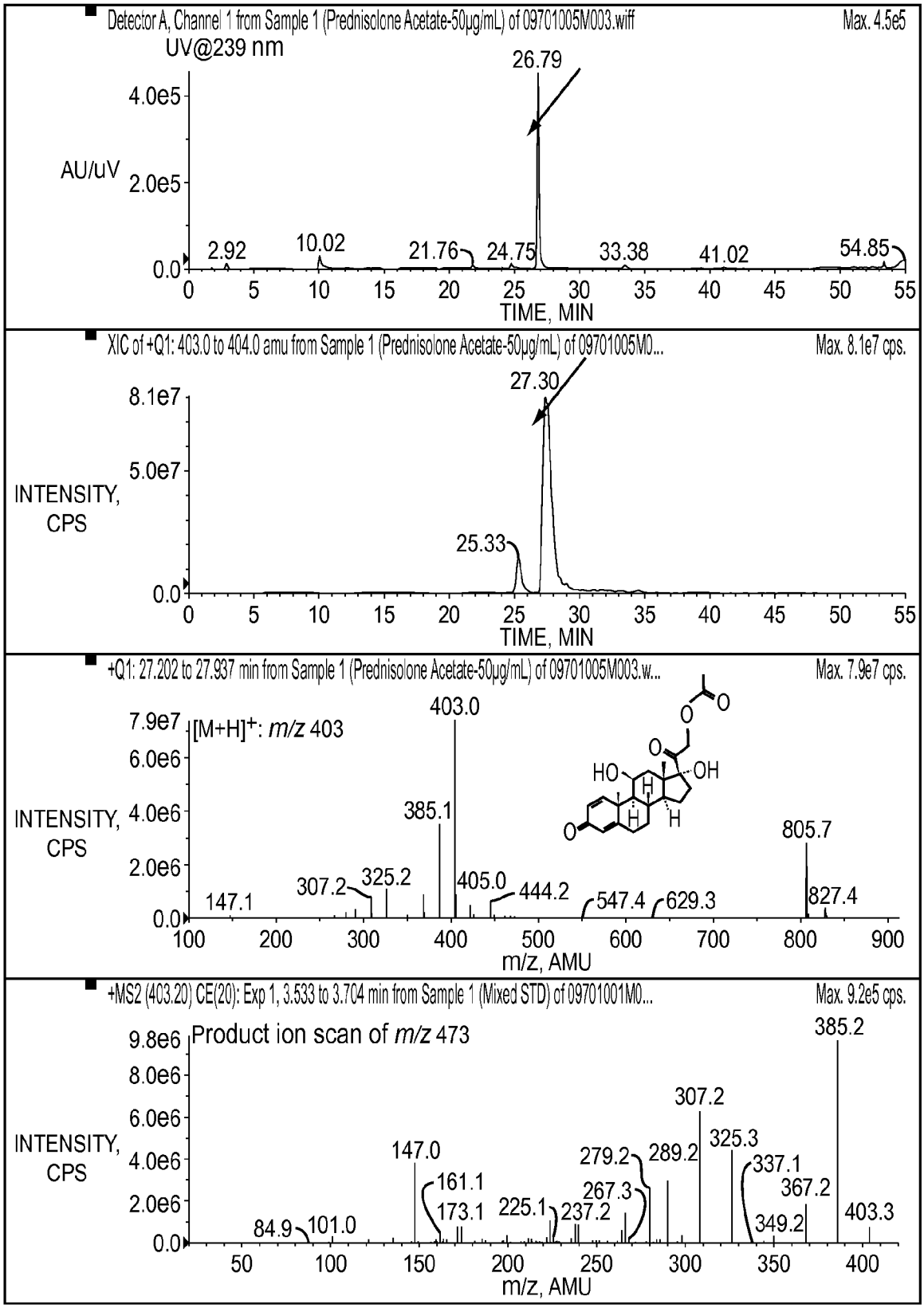


FIG. 2

HPLC-UV/(+)ESI-MS and MS/MS Spectral Data of Loteprednol Etabonate

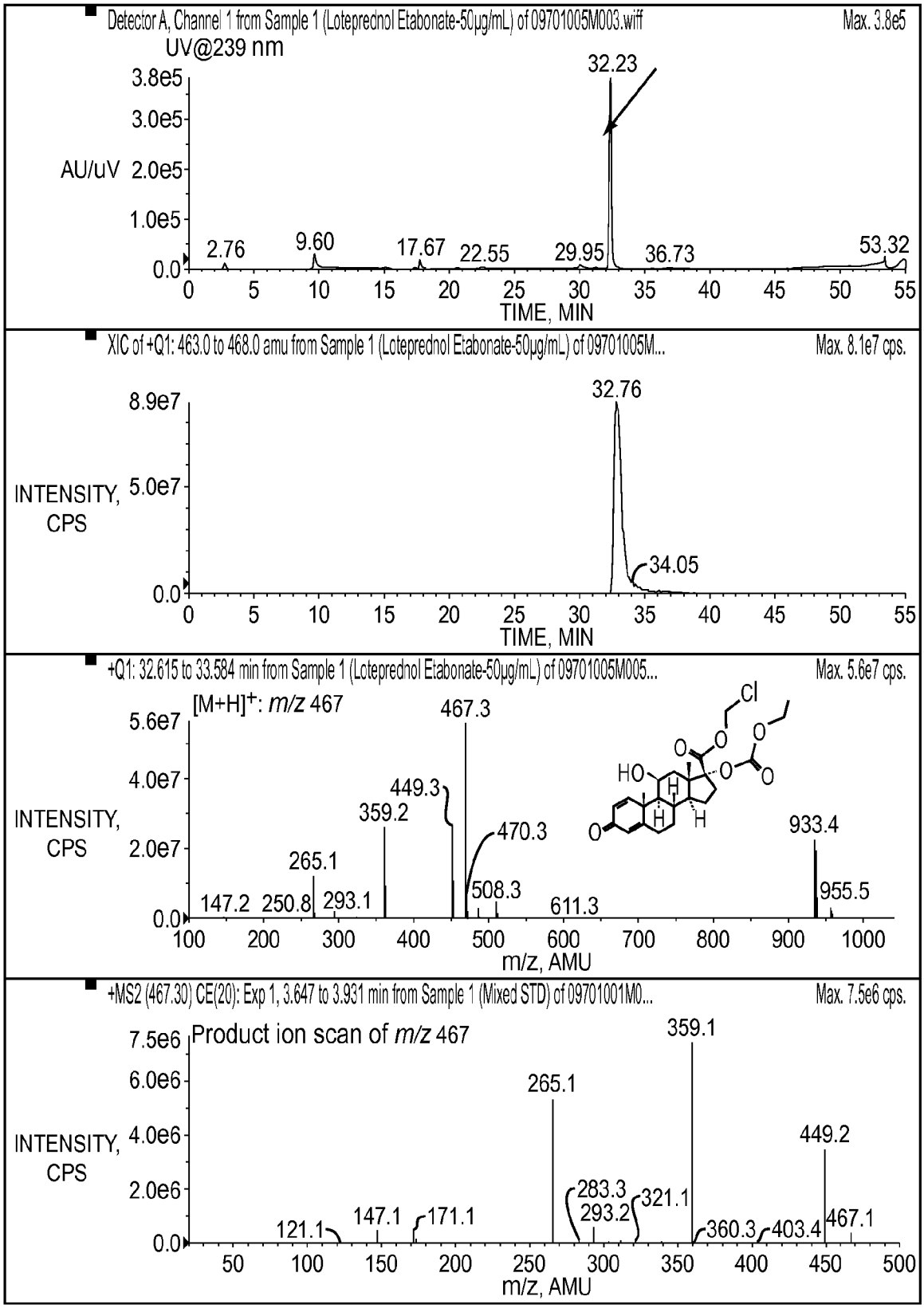


FIG. 3

HPLC-UV/(+)ESI-MS and MS/MS Spectral Data of Difluprednate

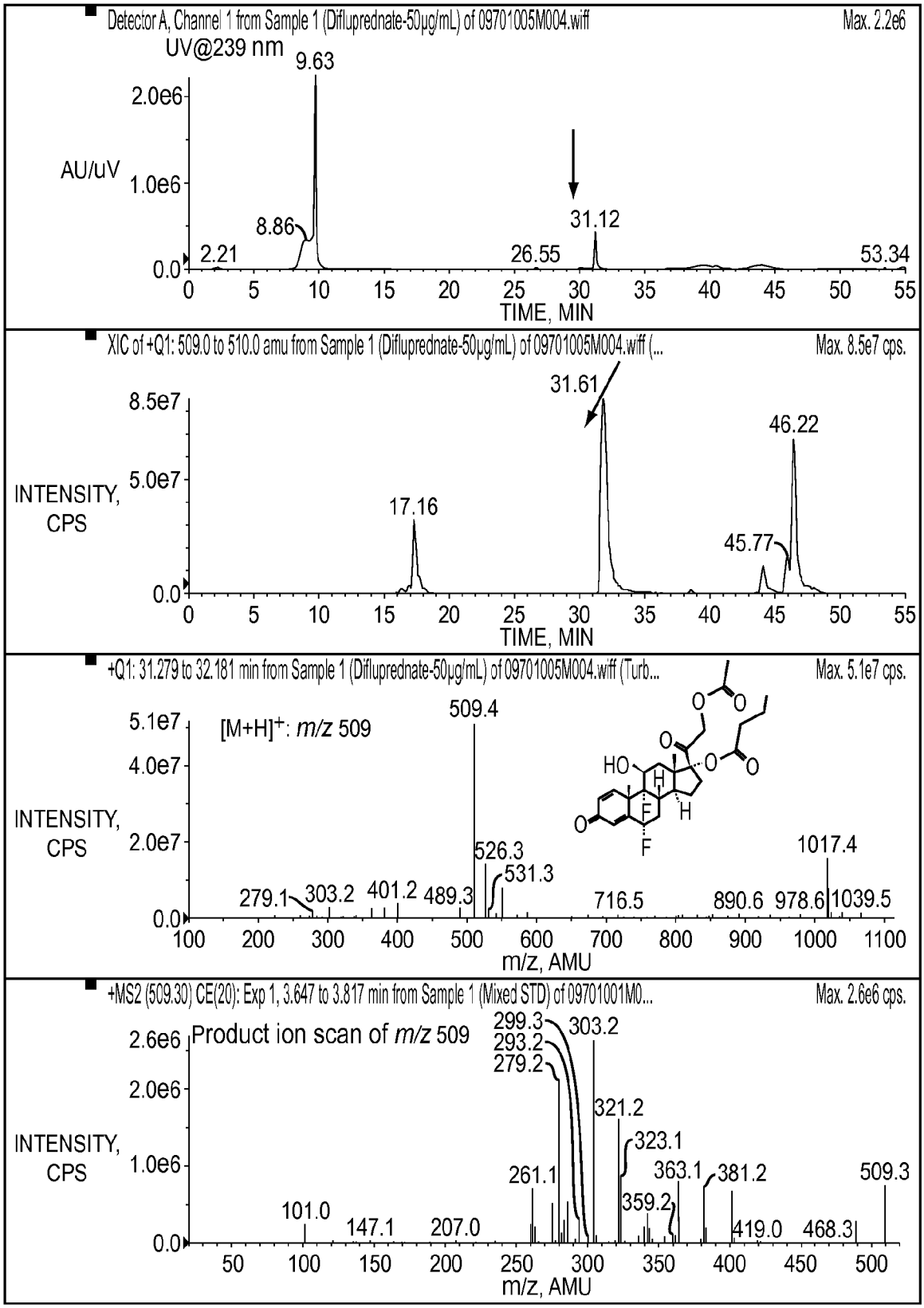


FIG. 4

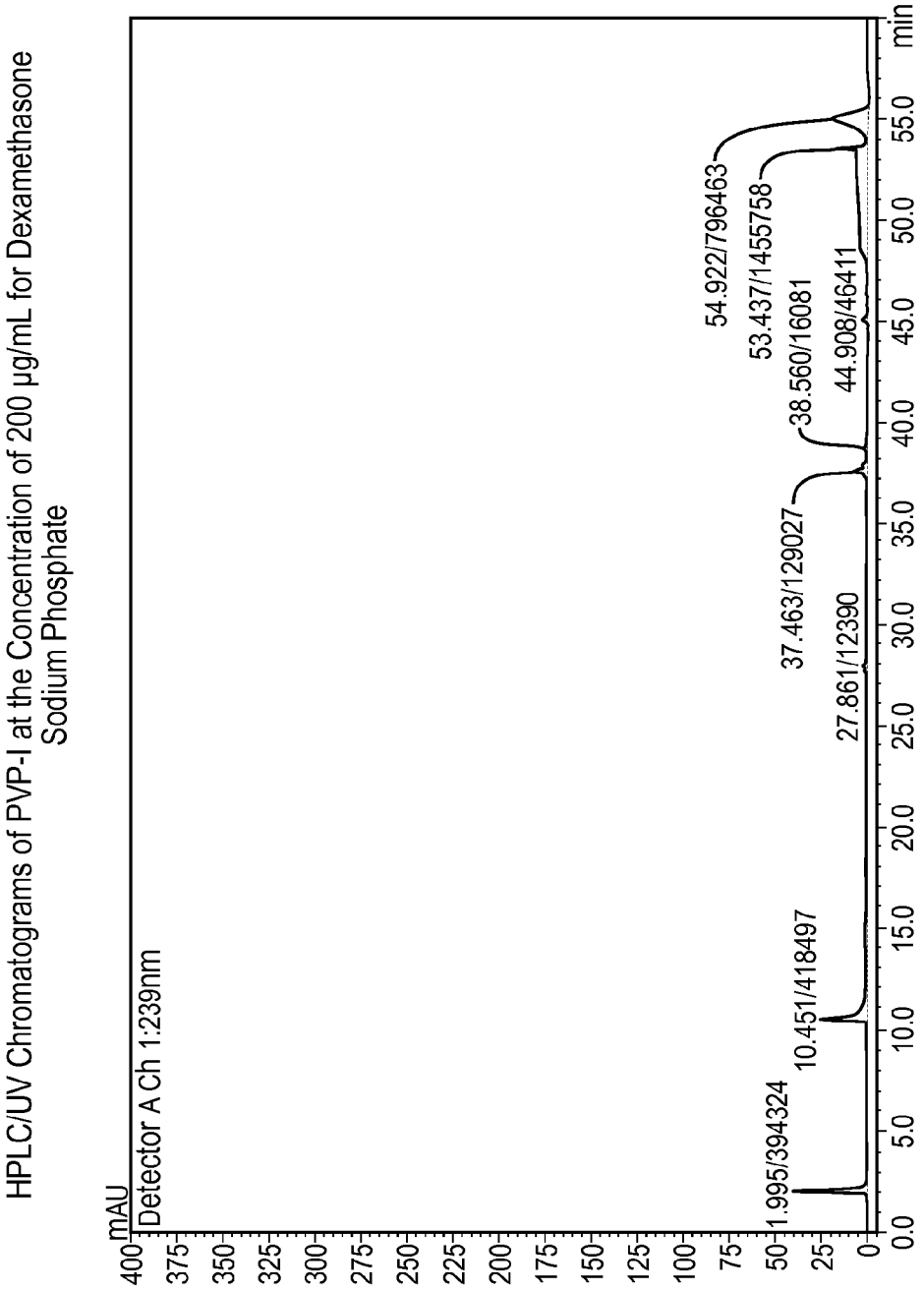

HPLC/UV Chromatograms of PVP-I at the Concentration of 200 µg/mL for Dexamethasone Sodium Phosphate
mAU
Detector A Ch 1:239nm
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
1.995/394324
10.451/418497
27.861/12390
37.463/129027
38.560/16081
44.908/46411
53.437/1455758
54.922/796463
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
min

FIG. 5

HPLC/UV Chromatograms of Dexamethasone Sodium Phosphate in PVP-I for Day 0

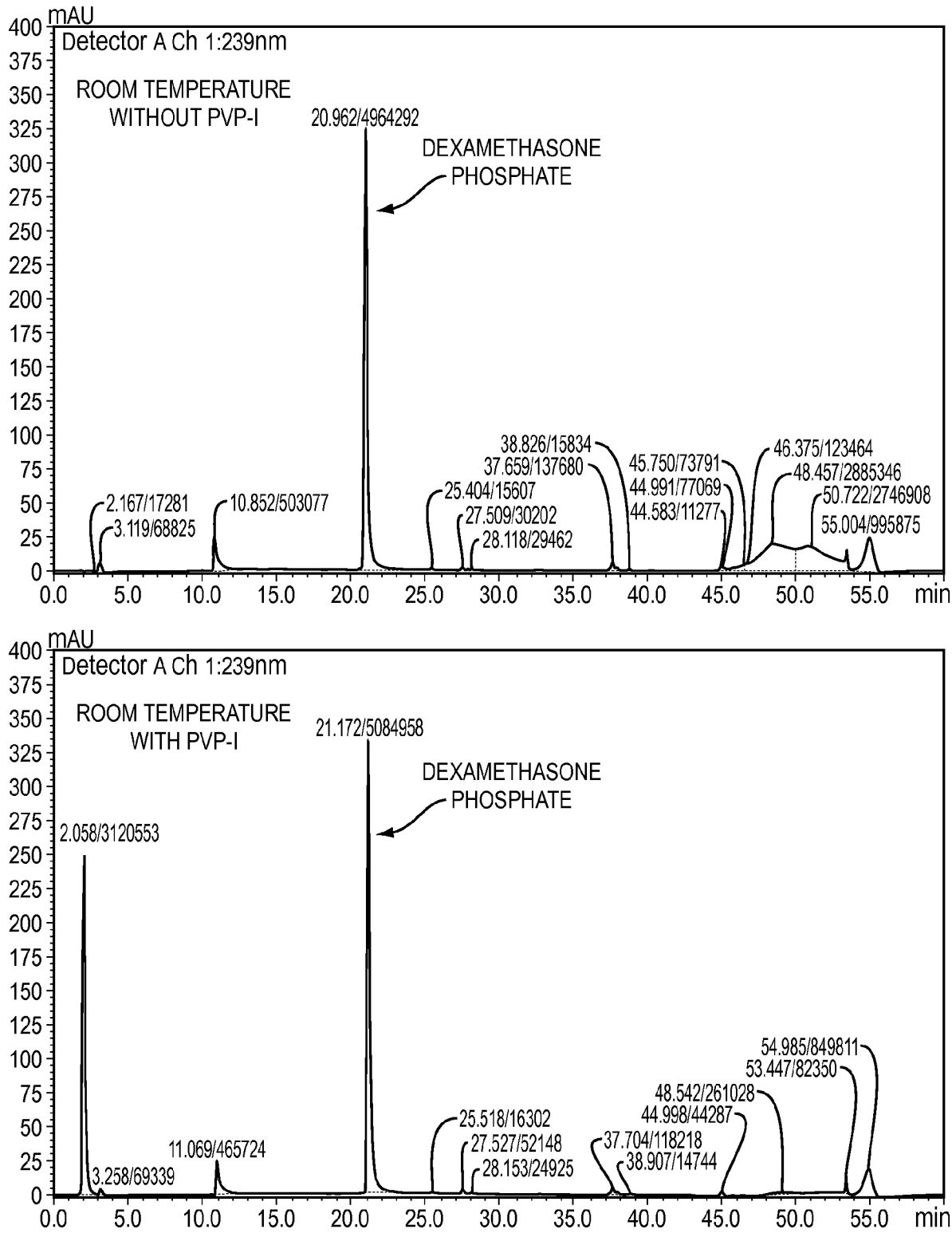


FIG. 6

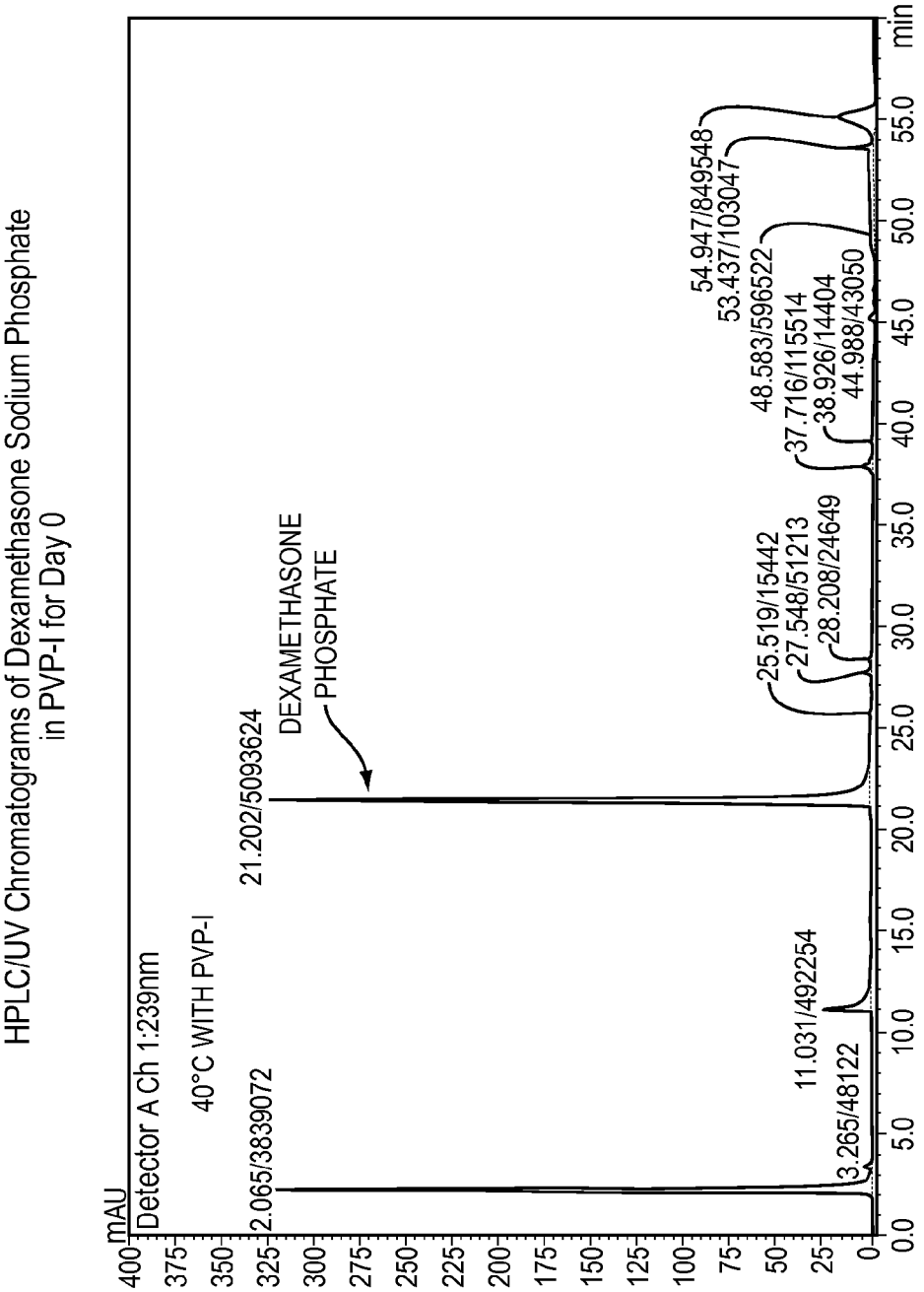

HPLC/UV Chromatograms of Dexamethasone Sodium Phosphate in PVP-I for Day 0
mAU
Detector A Ch 1:239nm
40°C WITH PVP-I
2.065/3839072
3.265/48122
11.031/492254
21.202/5093624
DEXAMETHASONE PHOSPHATE
25.519/15442
27.548/51213
28.208/24649
37.716/115514
38.926/14404
44.988/43050
48.583/596522
53.437/103047
54.947/849548
min

FIG. 7

HPLC/UV Chromatograms of Dexamethasone Sodium Phosphate
in PVP-I for Two Weeks

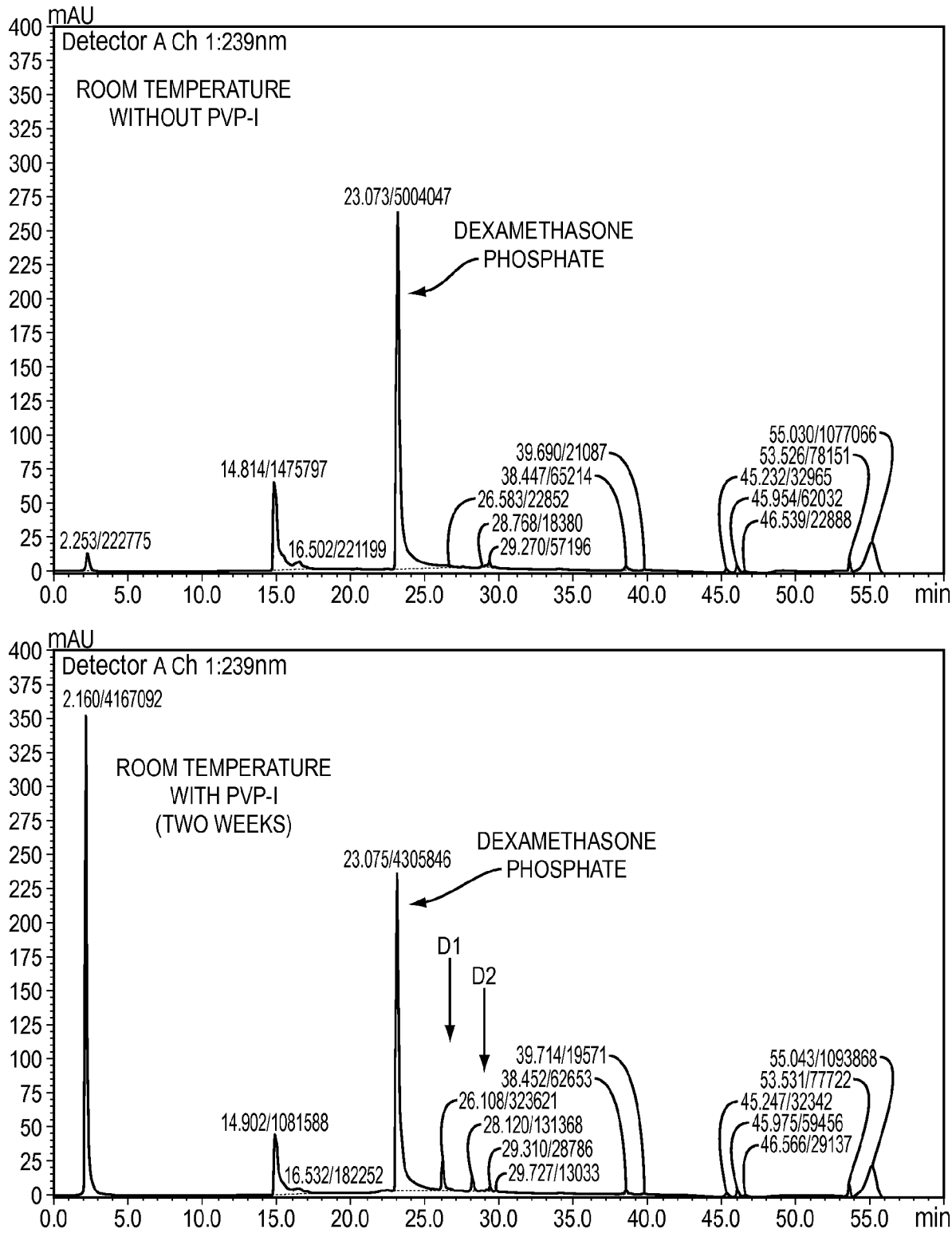


FIG. 8

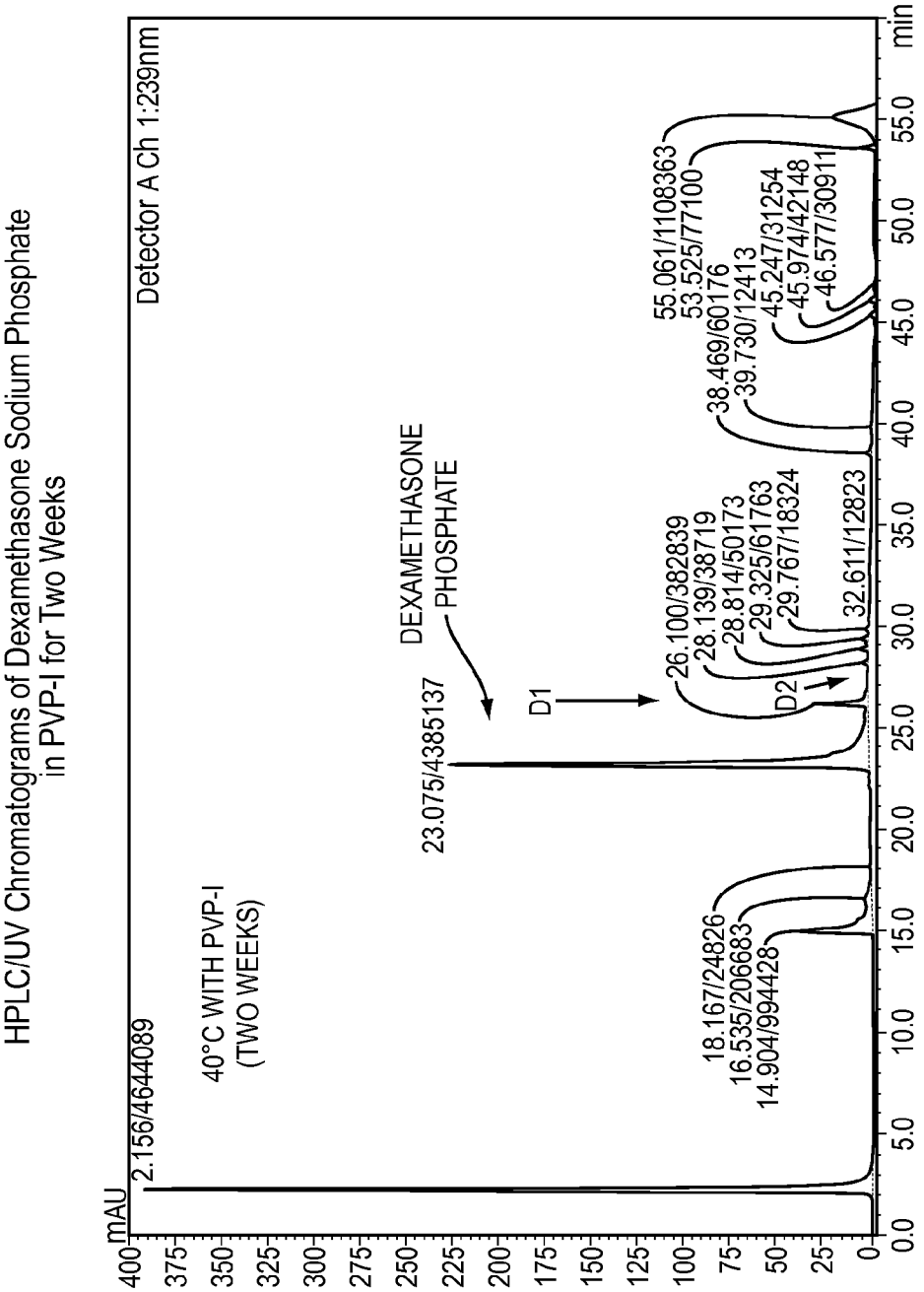

HPLC/UV Chromatograms of Dexamethasone Sodium Phosphate in PVP-I for Two Weeks
Detector A Ch 1:239nm
40°C WITH PVP-I (TWO WEEKS)
mAU
min
2.156/4644089
23.075/4385137
DEXAMETHASONE PHOSPHATE
D1
D2
18.167/24826
16.535/206683
14.904/994428
26.100/382839
28.139/38719
28.814/50173
29.325/61763
29.767/18324
32.611/12823
38.469/60176
39.730/12413
45.247/31254
45.974/42148
46.577/30911
53.525/77100
55.061/1108363

FIG. 9

HPLC/UV Chromatograms of Dexamethasone Sodium Phosphate in PVP-I for One Month

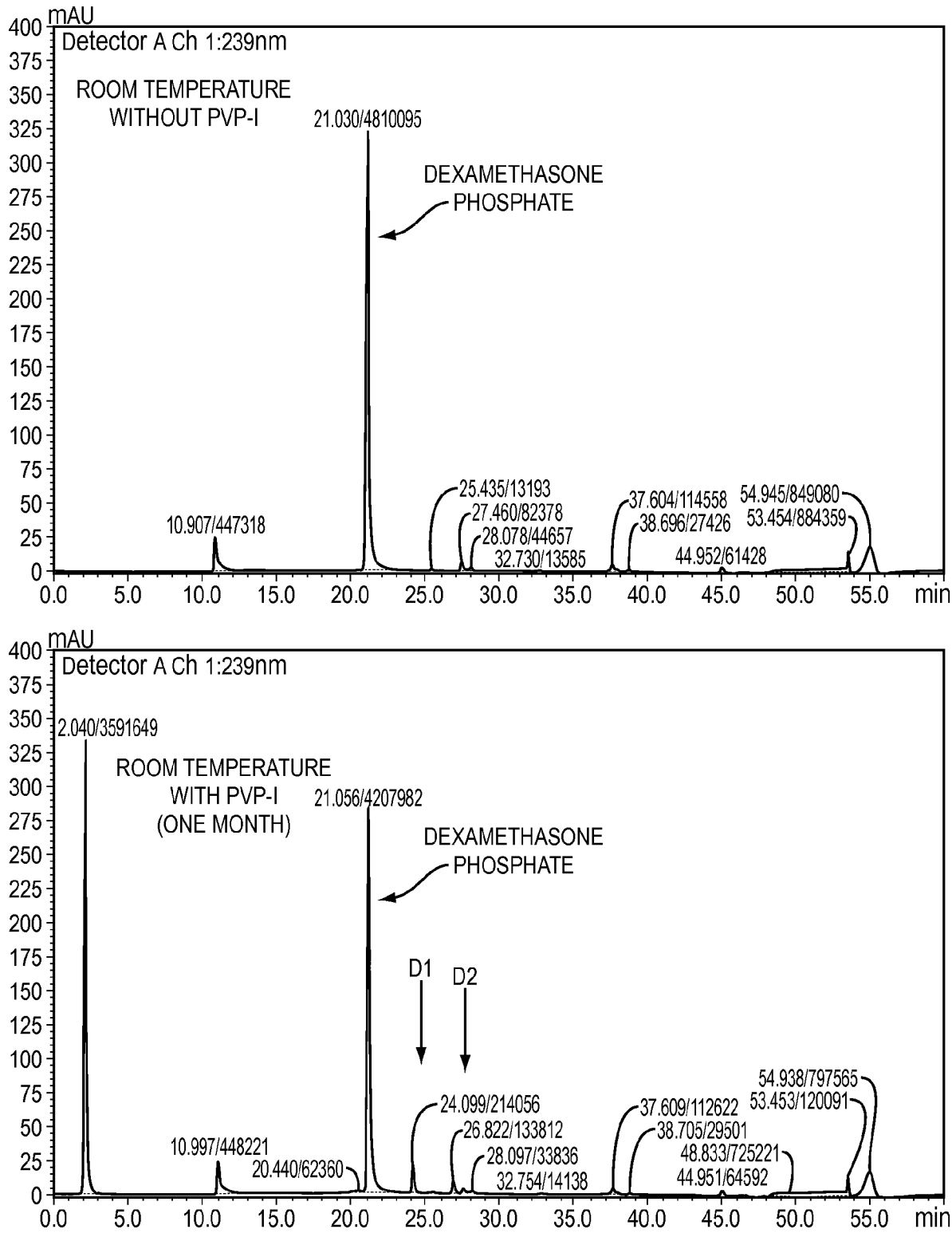


FIG. 10

HPLC/UV Chromatograms of Dexamethasone Sodium Phosphate in PVP-I for One Month

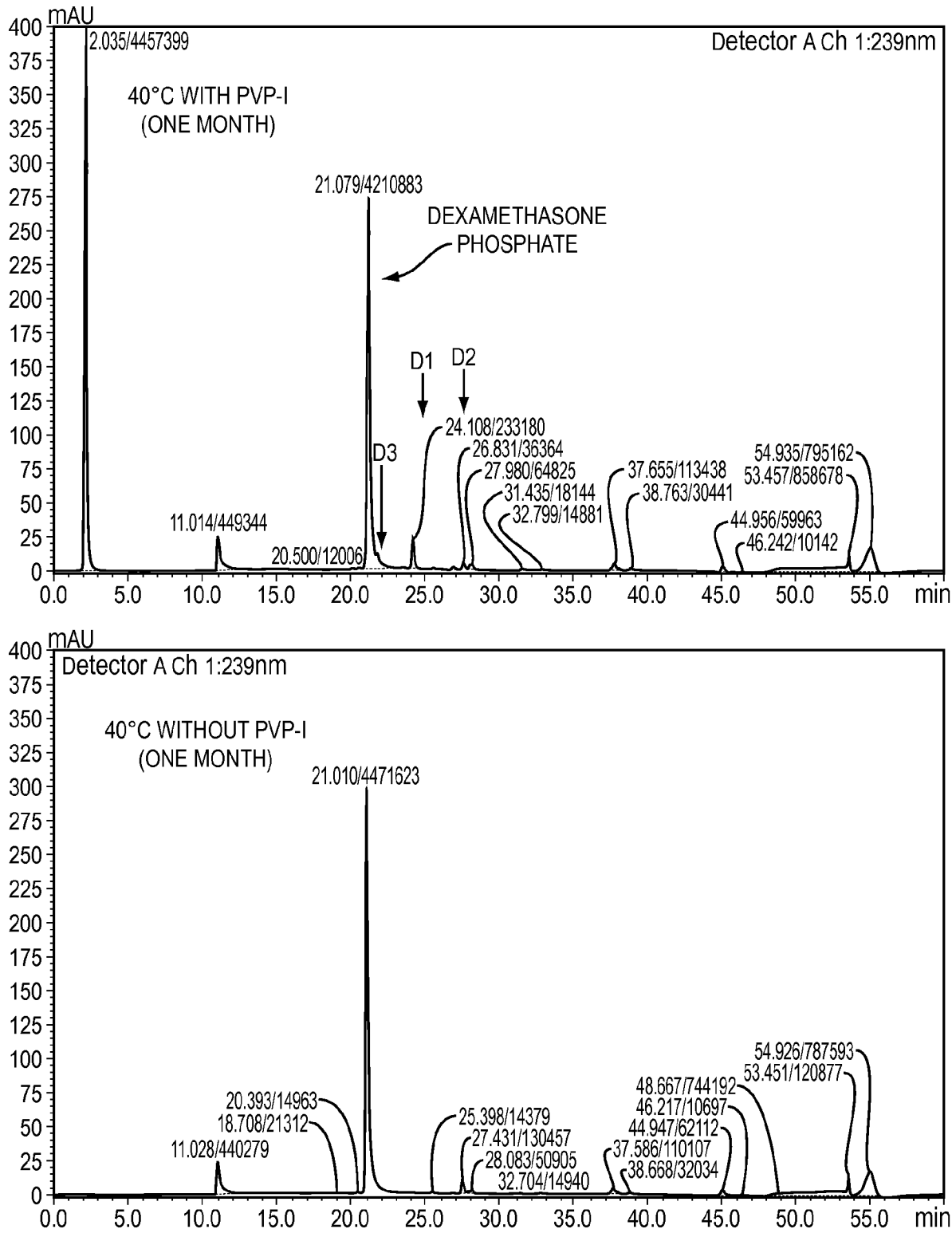


FIG. 11

HPLC/UV Chromatograms (Expanded) of Dexamethasone Sodium Phosphate in PVP-I for One Month

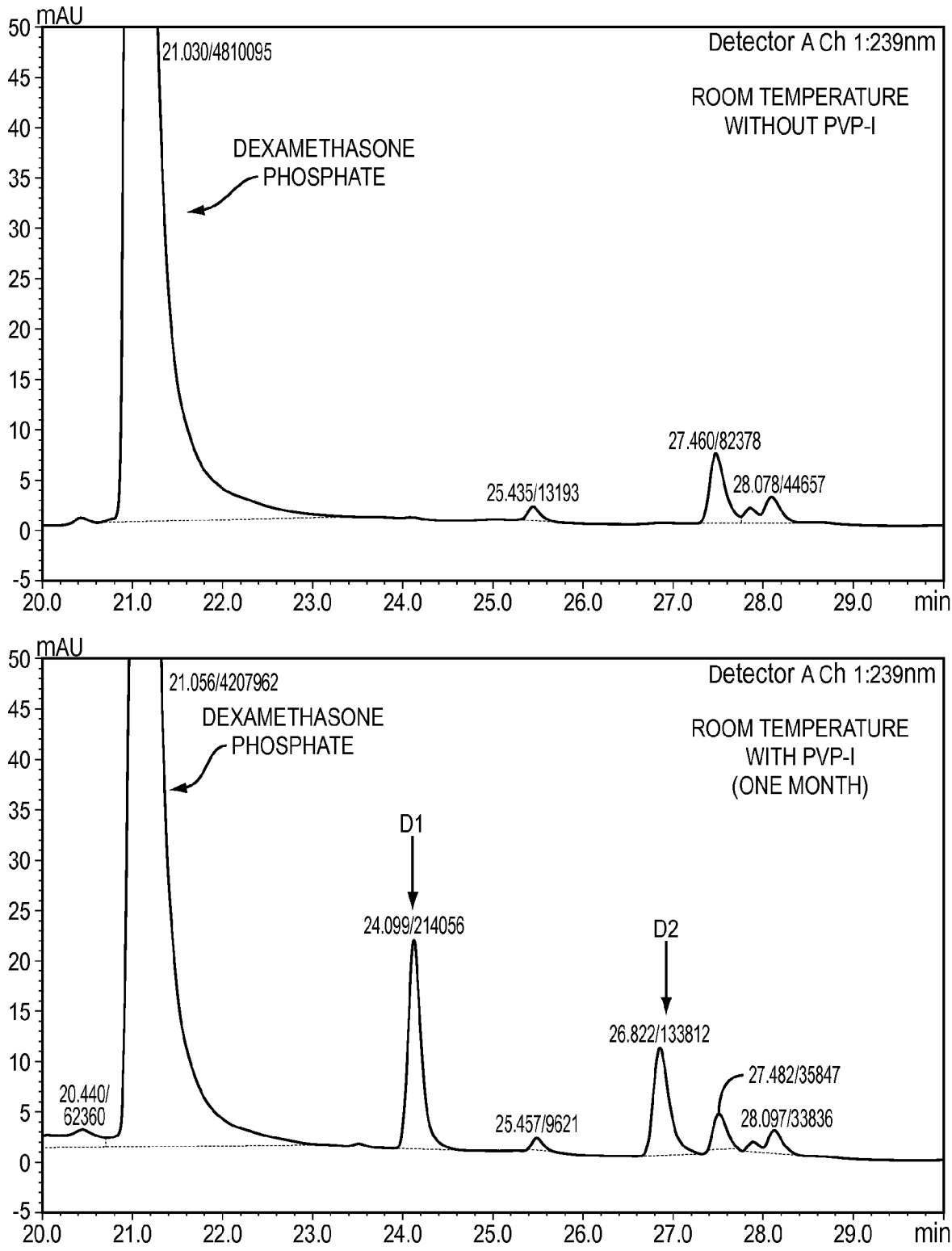


FIG. 12

HPLC/UV Chromatograms (Expanded) of Dexamethasone Sodium Phosphate in PVP-I for One Month

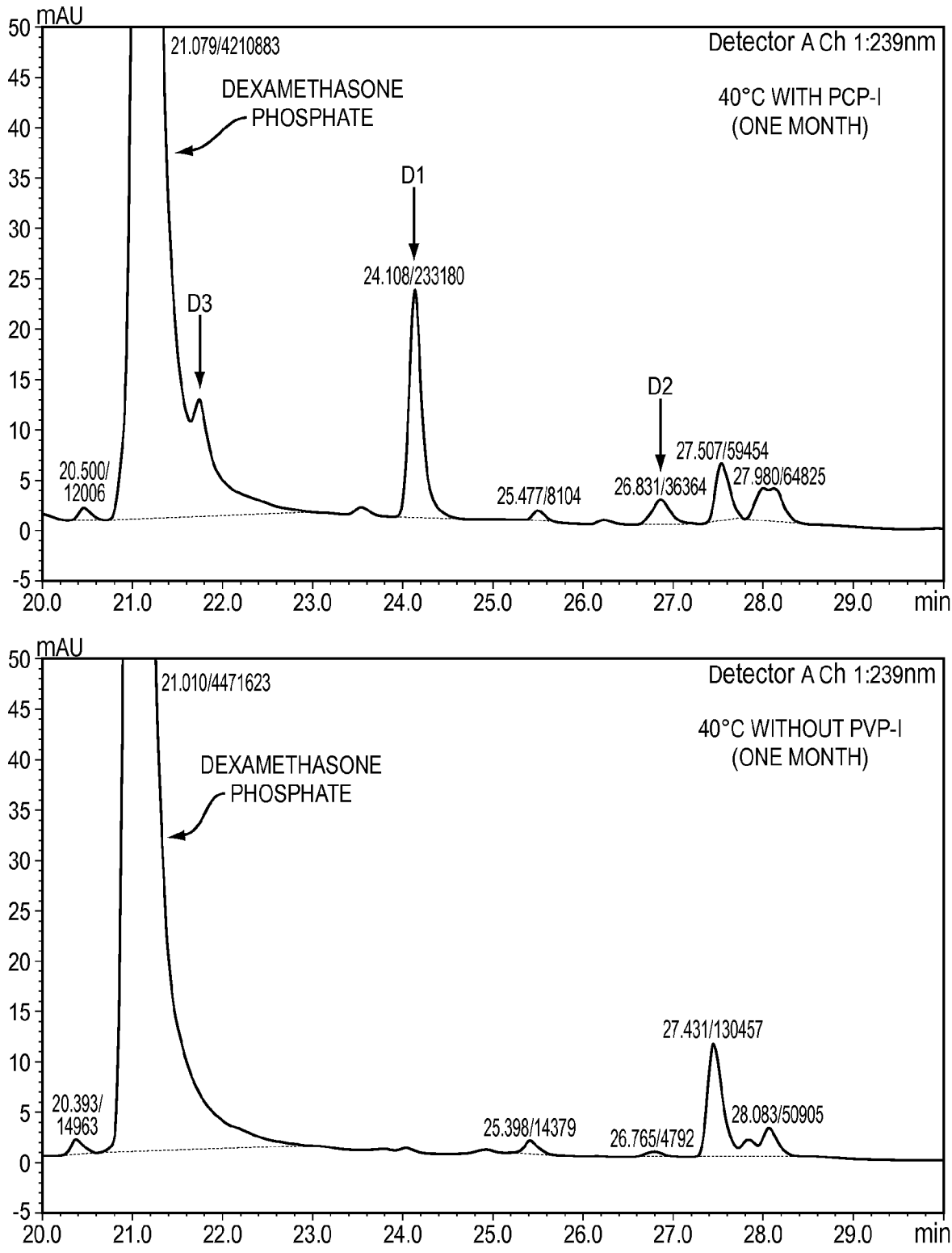


FIG. 13

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Mass Ion Chromatograms (MRM Mode) of Dexamethasone Sodium Phosphate in Reference Standard Samples

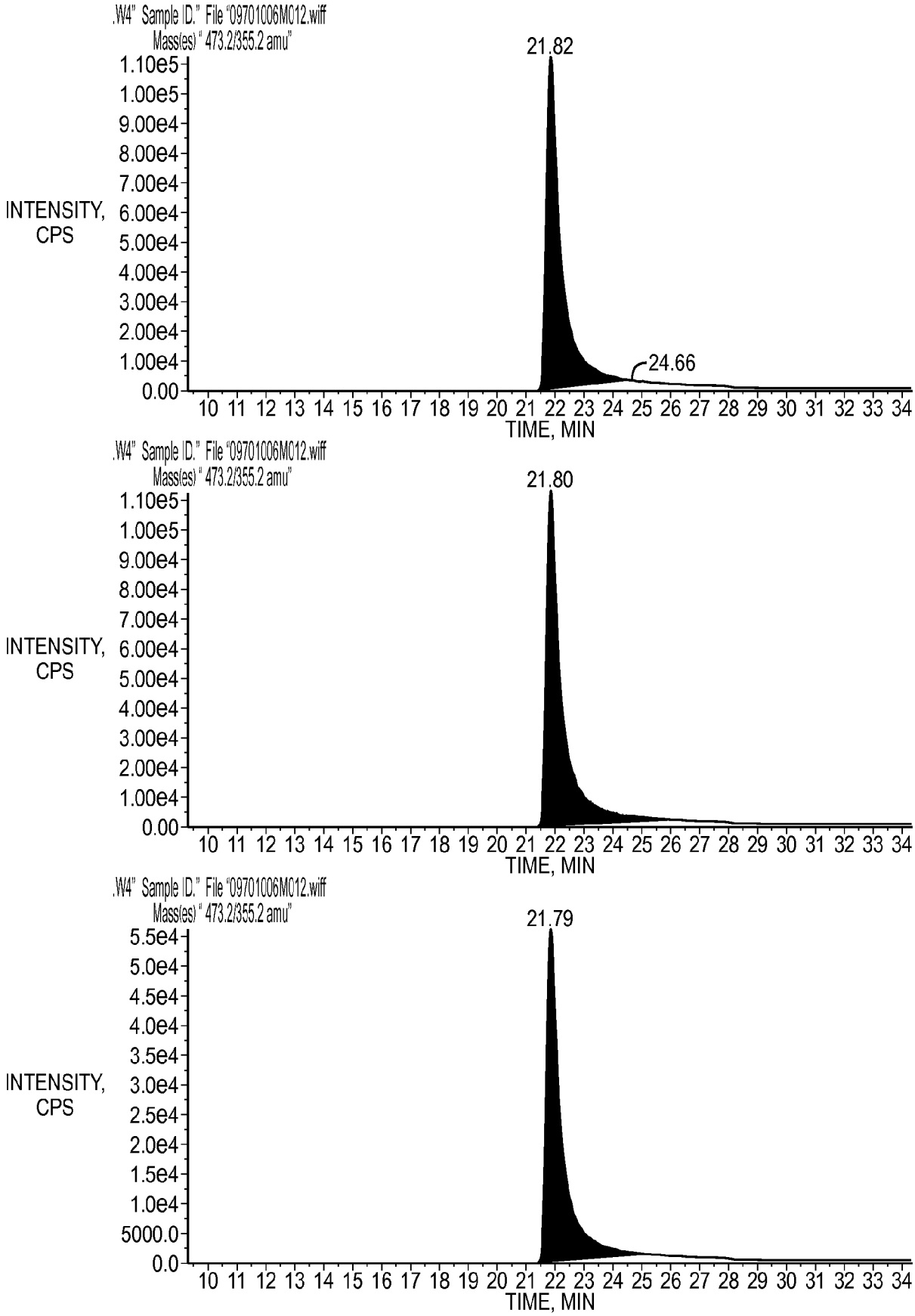

FIG. 14

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Mass Ion Chromatograms (MRM Mode) of Dexamethasone Sodium Phosphate in One Month Room Temperature Stability Sample in the Presence of PVP-I

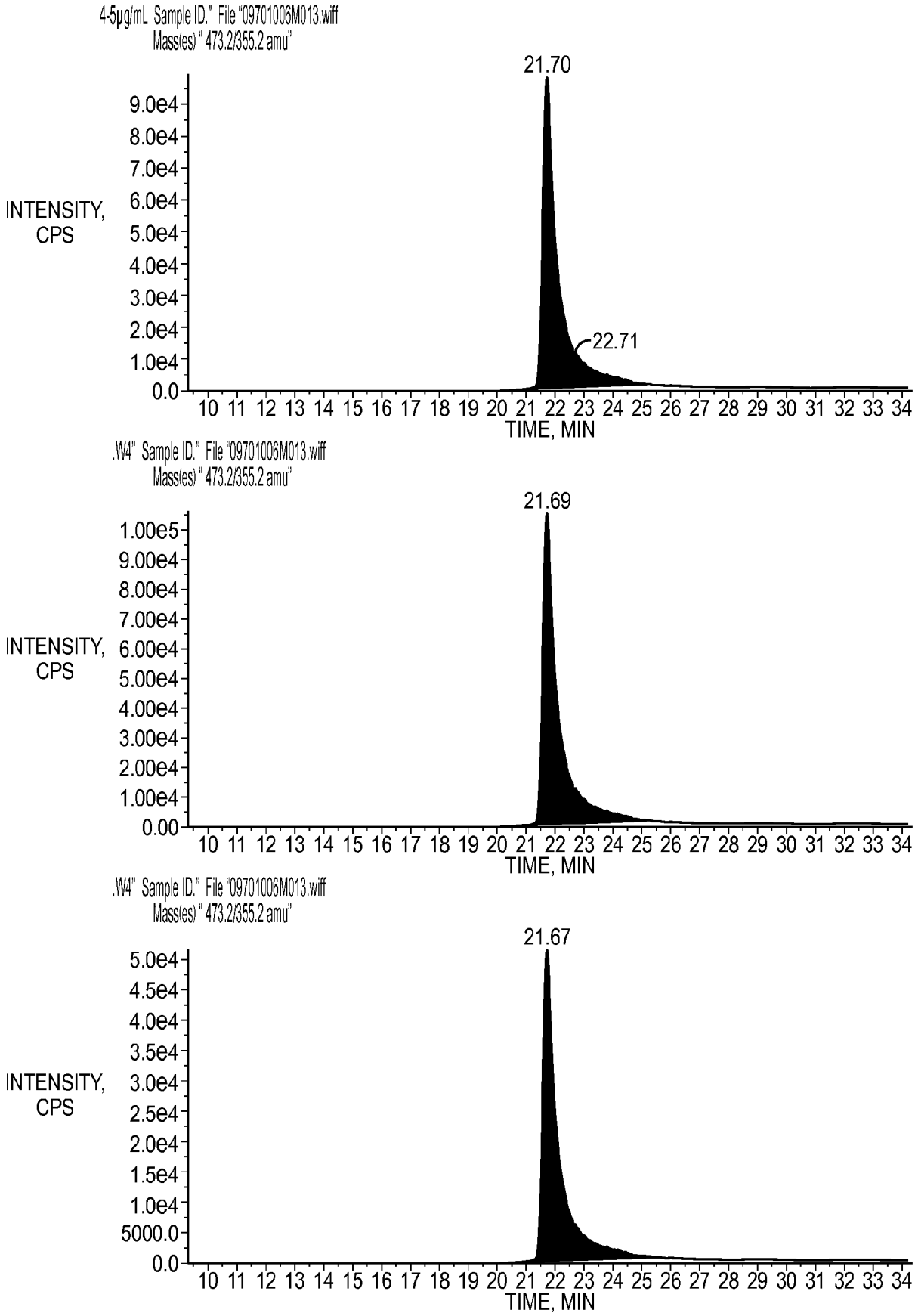

FIG. 15

Mass Ion Chromatograms (MRM Mode) of Dexamethasone Sodium Phosphate in One Month 40°C Stability Sample in the Presence of PVP-I

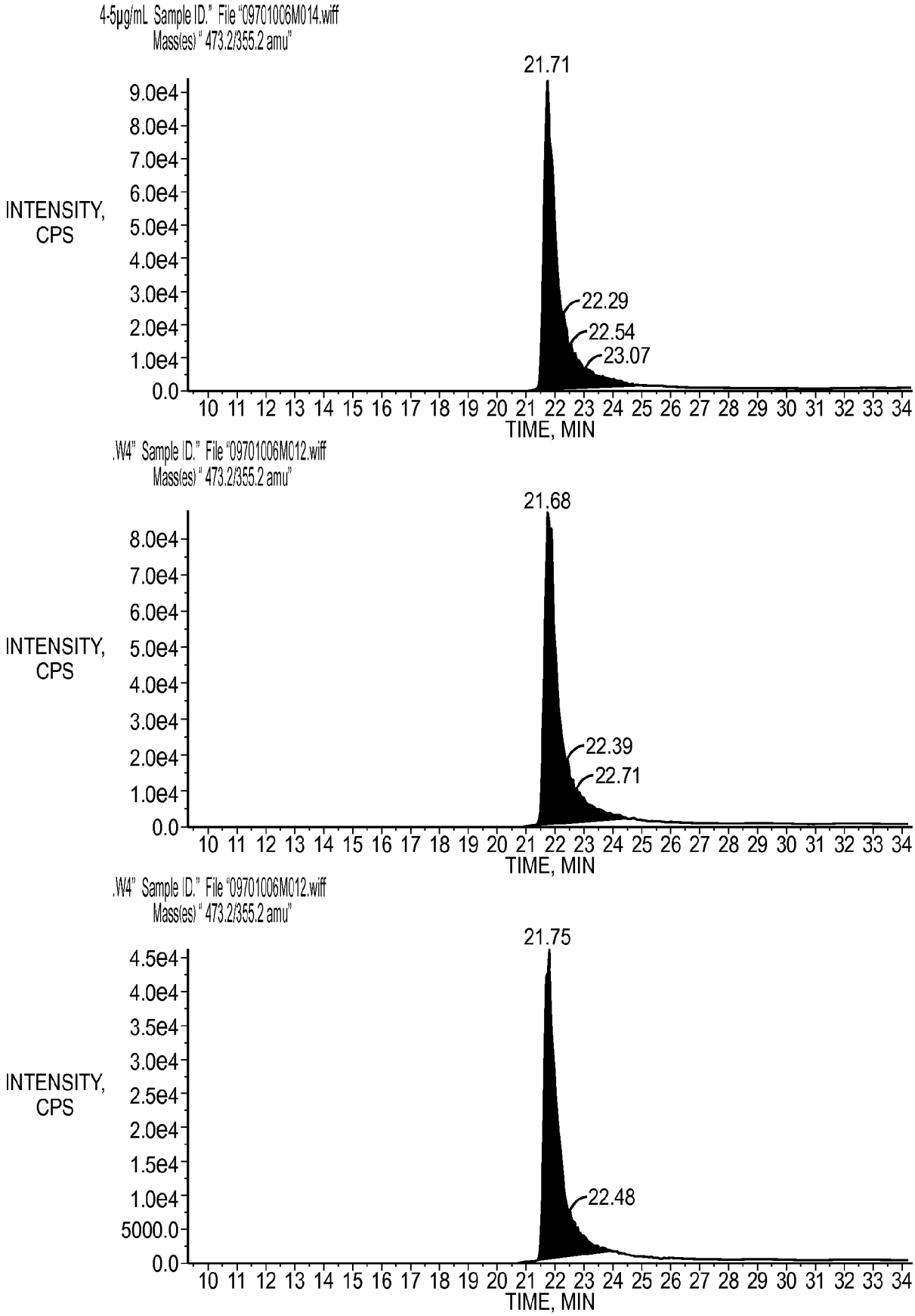

FIG. 16

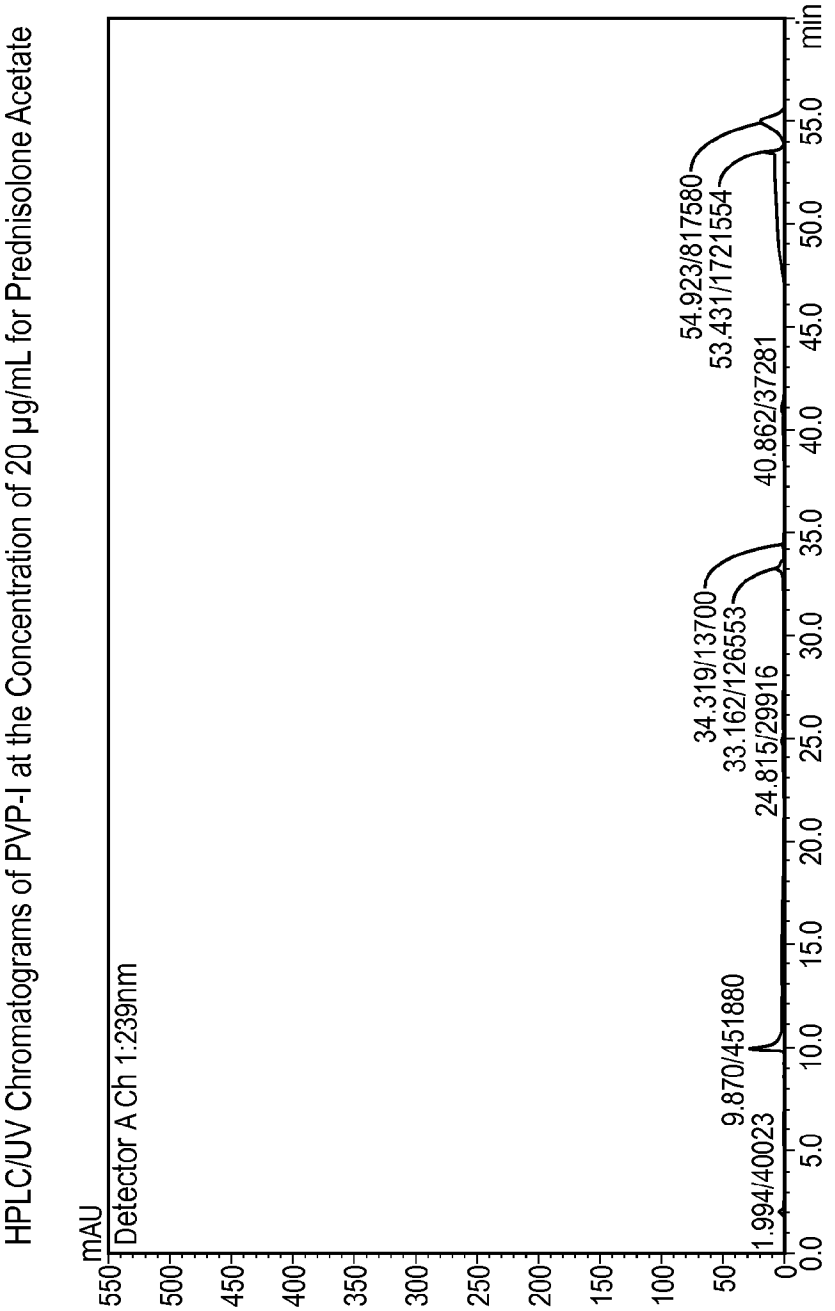
HPLC/UV Chromatograms of PVP-I at the Concentration of 20 µg/mL for Prednisolone Acetate
mAU
Detector A Ch 1:239nm
550
500
450
400
350
300
250
200
150
100
50
0
1.994/40023
9.870/451880
24.815/29916
33.162/126553
34.319/13700
40.862/37281
53.431/1721554
54.923/817580
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
min

FIG. 17

HPLC/UV Chromatograms of Prednisolone Acetate in PVP-I for Day 0

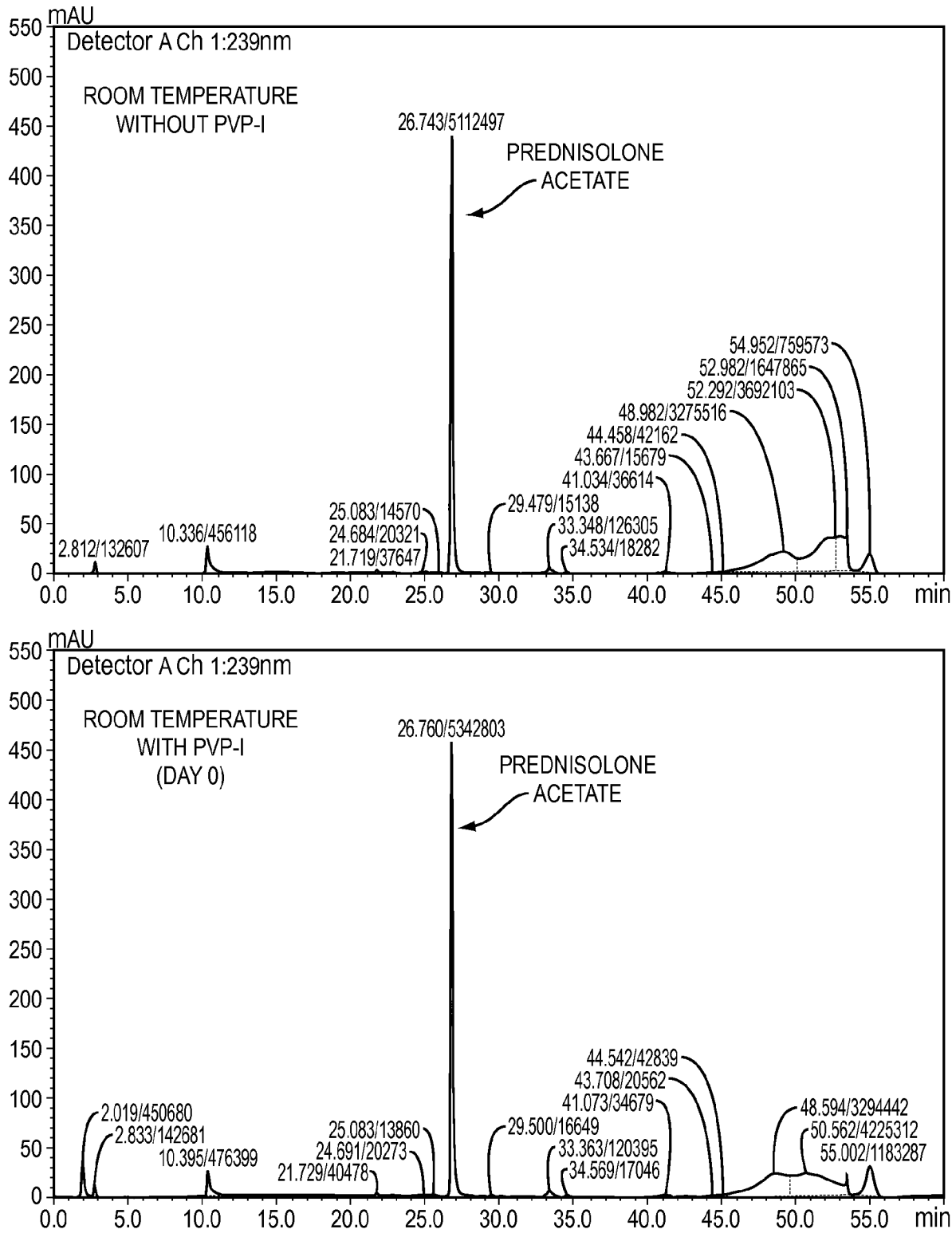

FIG. 18

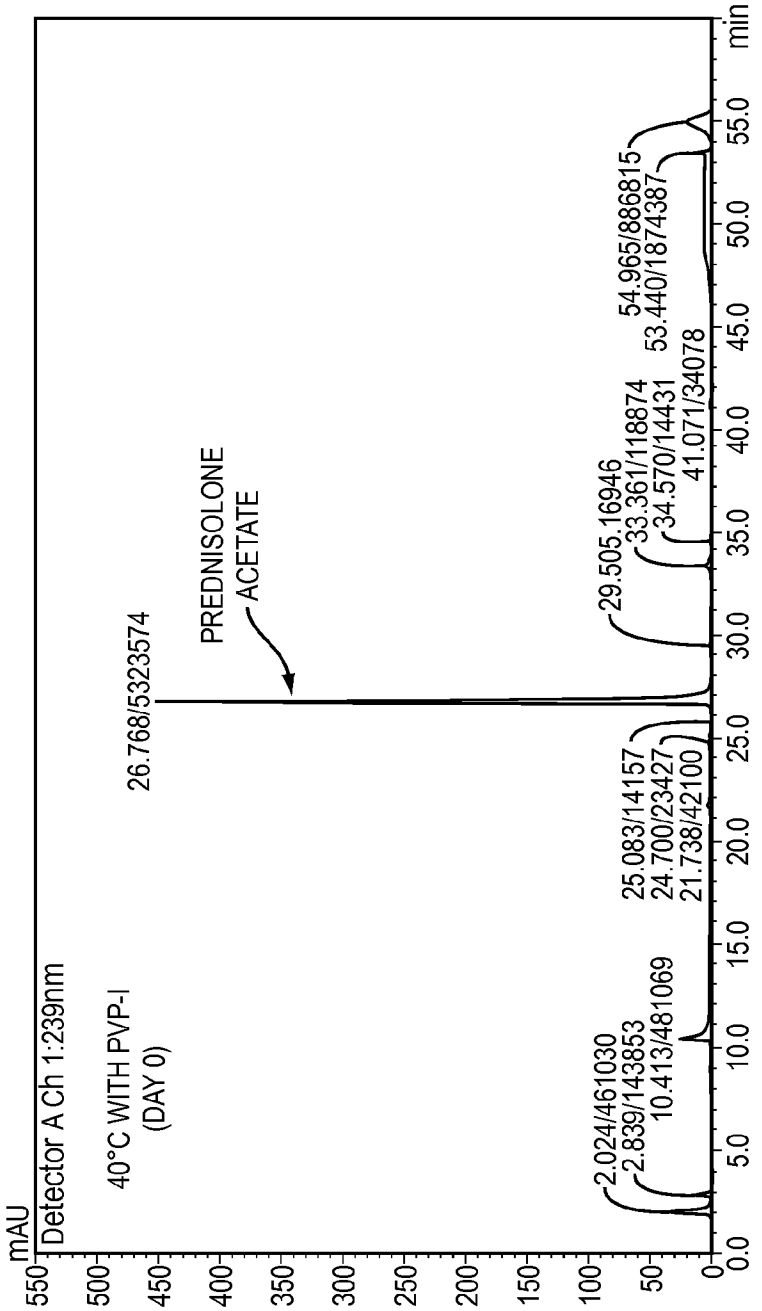
HPLC/UV Chromatograms of Prednisolone Acetate in PVP-I for Day 0
Detector A Ch 1:239nm
40°C WITH PVP-I
(DAY 0)
mAU
550
500
450
400
350
300
250
200
150
100
50
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
min
2.024/461030
2.839/143853
10.413/481069
21.738/42100
24.700/23427
25.083/14157
26.768/5323574
PREDNISOLONE
ACETATE
29.505.16946
33.361/118874
34.570/14431
41.071/34078
53.440/1874387
54.965/886815

FIG. 19

HPLC/UV Chromatograms of Prednisolone Acetate in PVP-I for Two Weeks

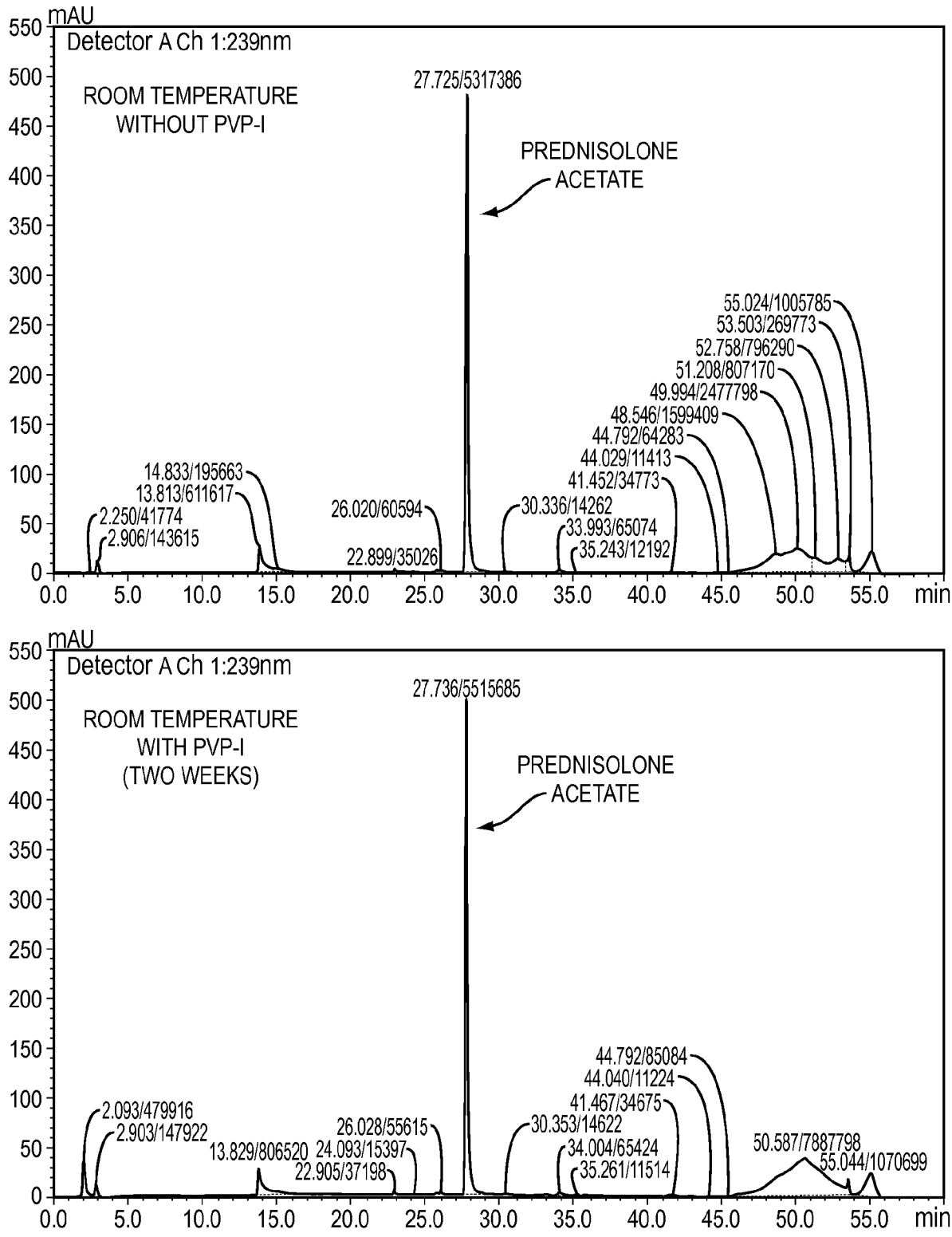

FIG. 20

HPLC/UV Chromatograms of Prednisolone Acetate in PVP-I for Two Weeks

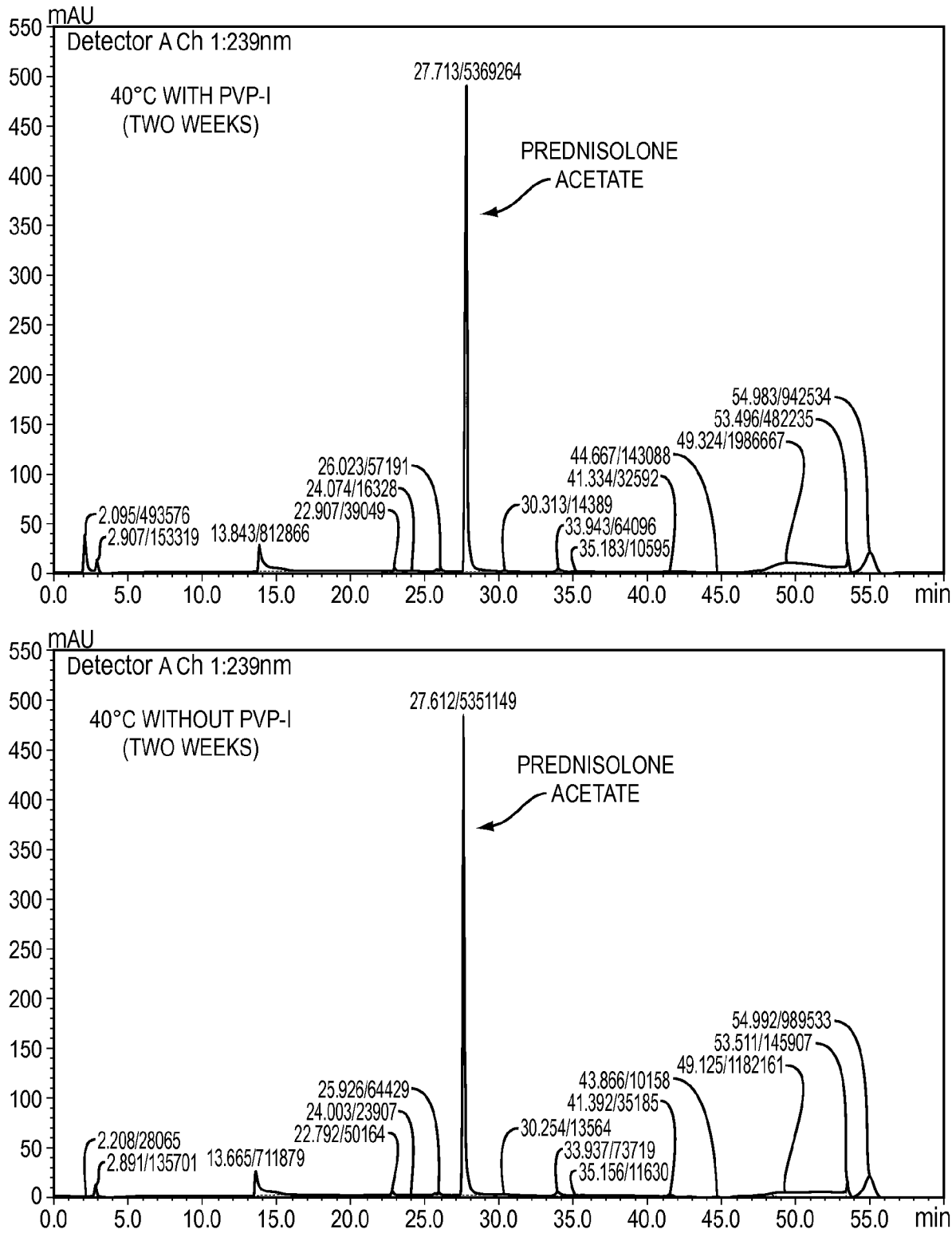

FIG. 21

HPLC/UV Chromatograms of Prednisolone Acetate in PVP-I for One Month

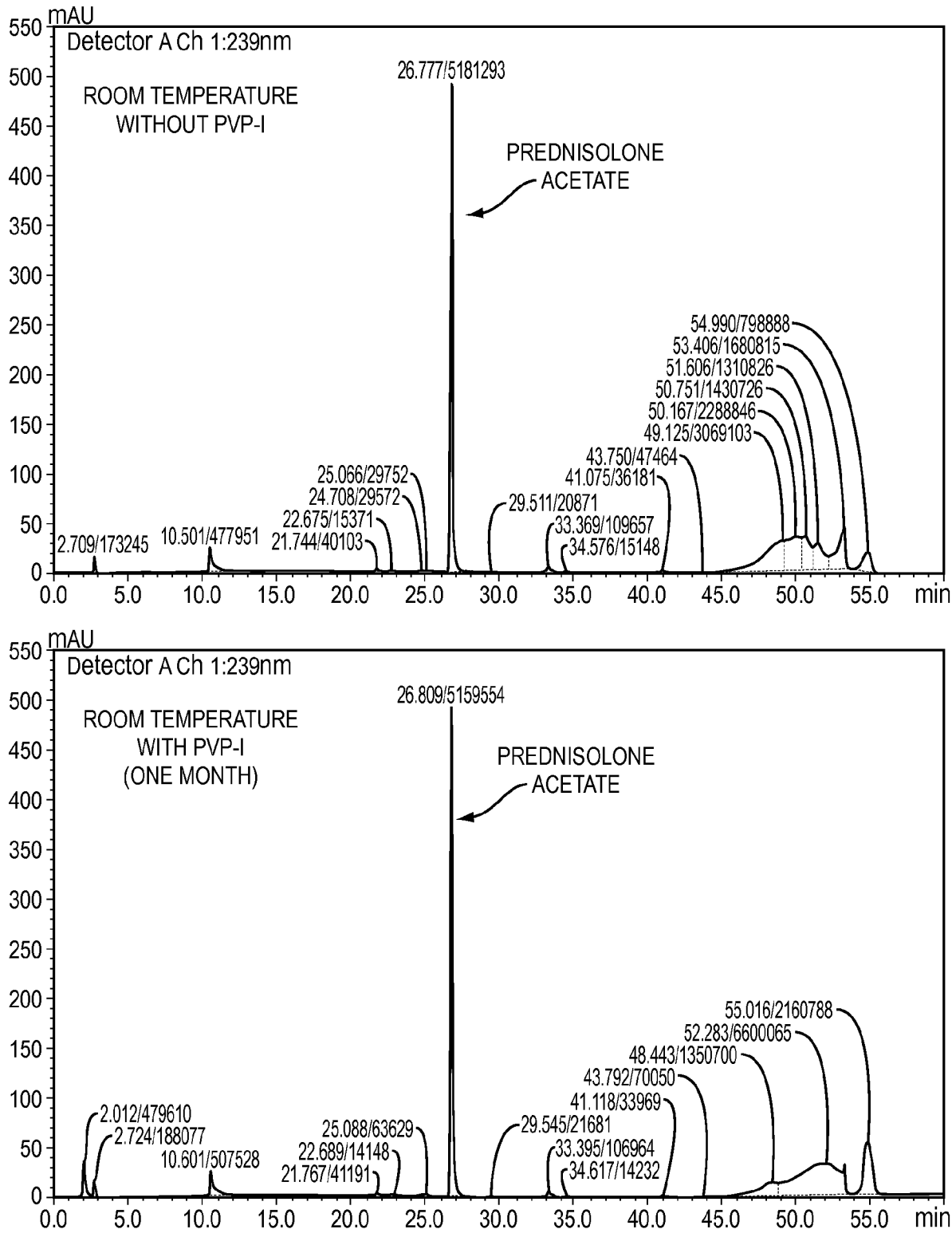

FIG. 22

HPLC/UV Chromatograms of Prednisolone Acetate in PVP-I for One Month

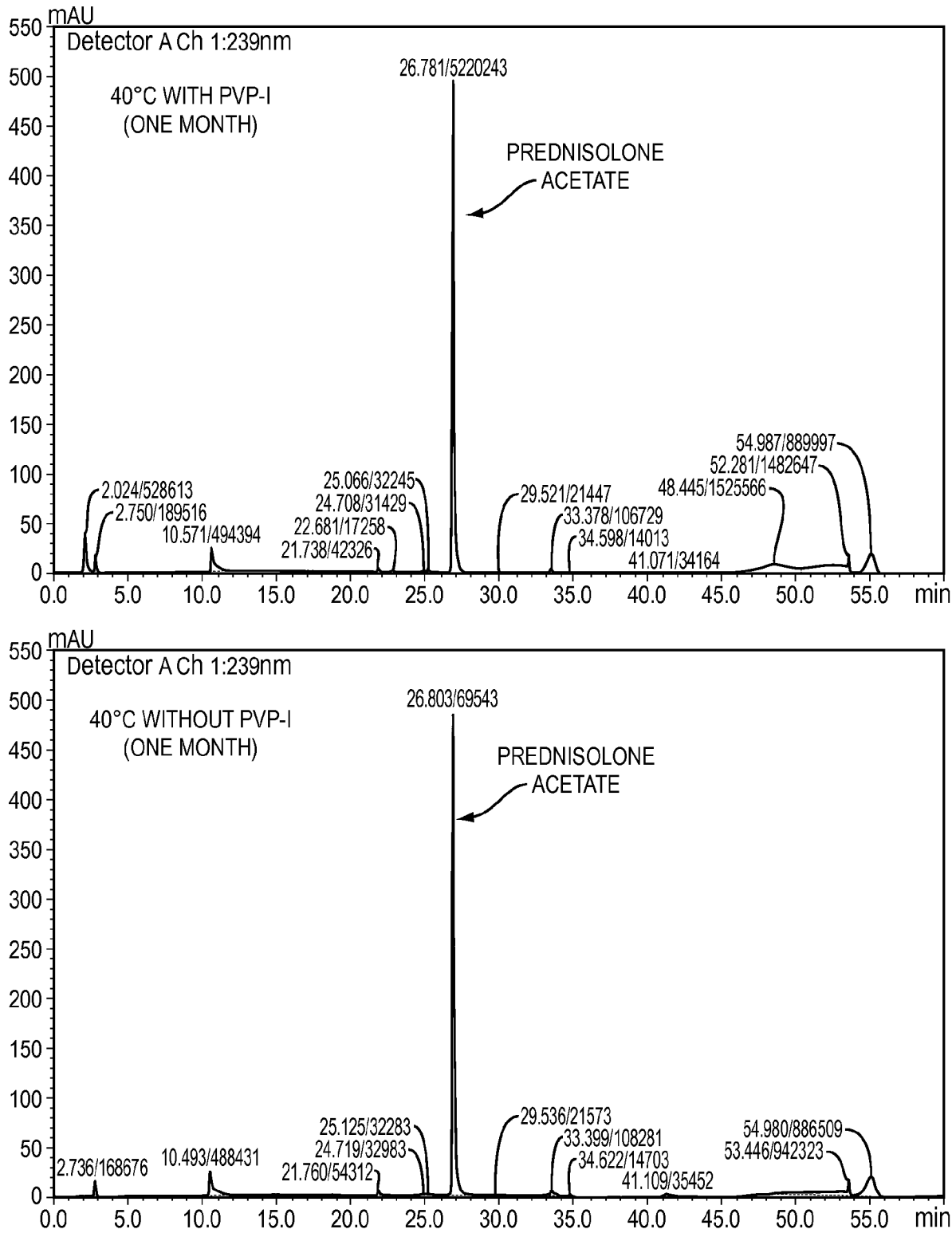

FIG. 23

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Mass Ion Chromatograms (MRM Mode) of Prednisolone Acetate in Reference Standard Samples

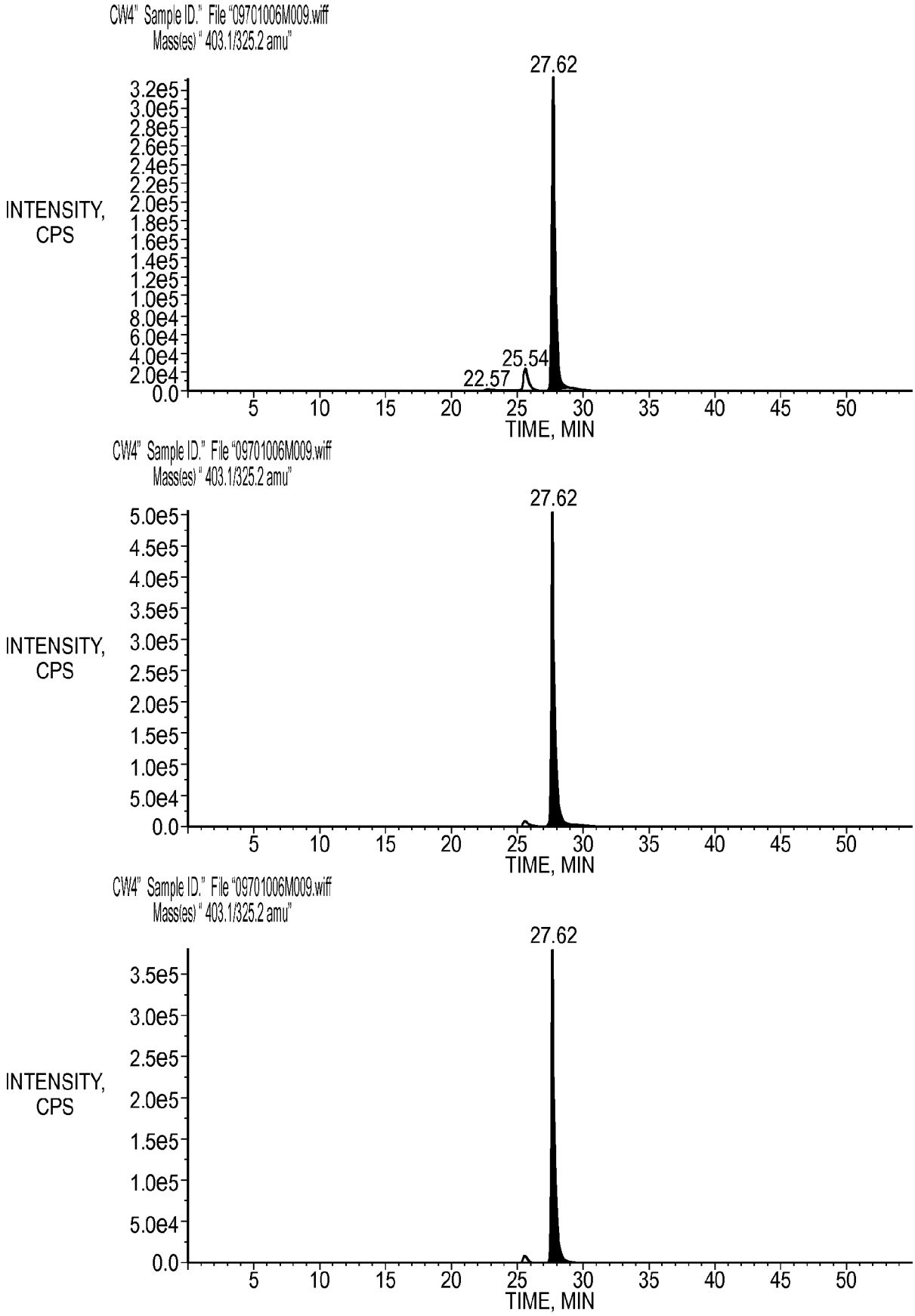

FIG. 24

Mass Ion Chromatograms (MRM Mode) of Prednisolone Acetate in One Month Room Temperature Stability Sample in the Presence of PVP-1

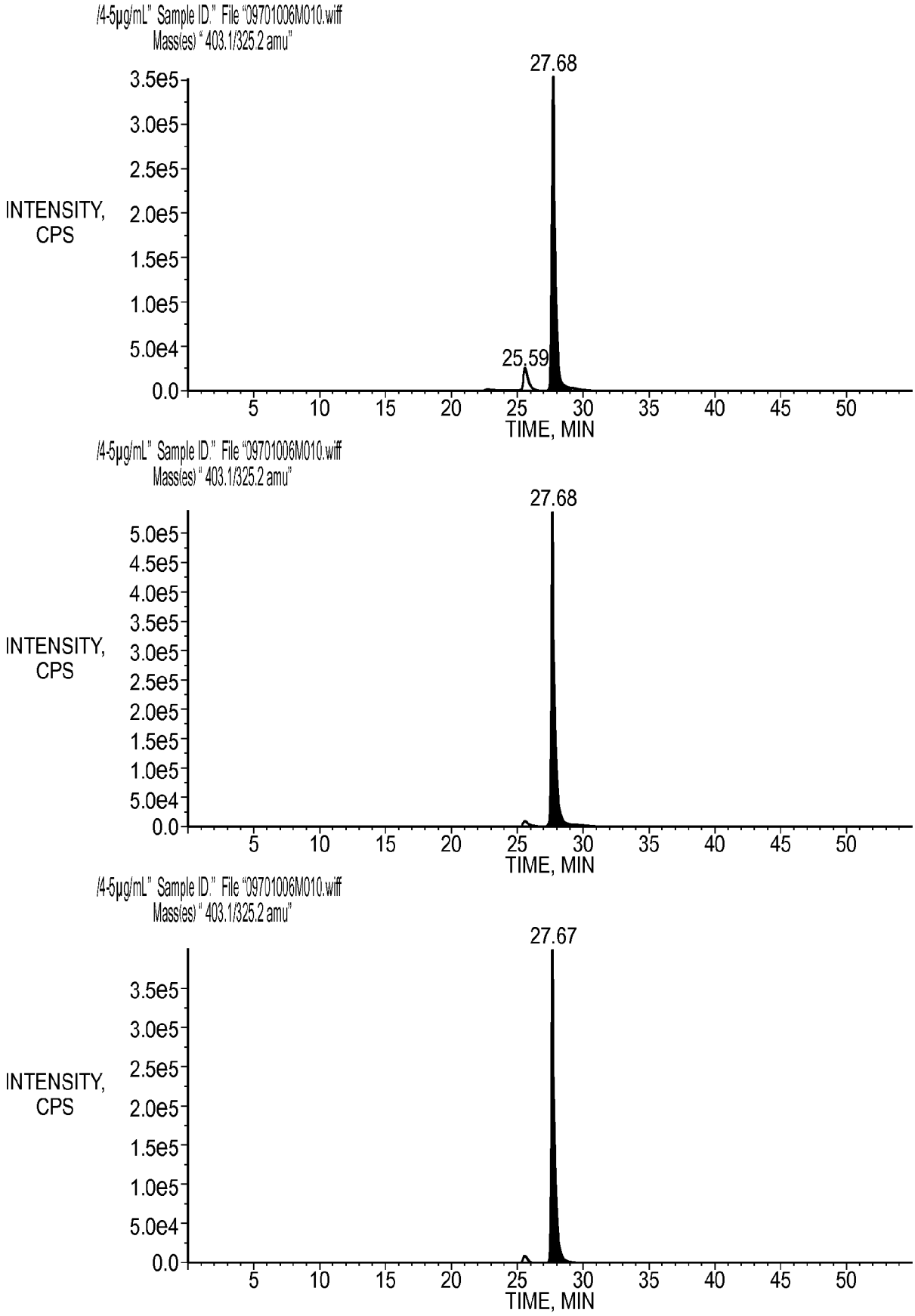

FIG. 25

Mass Ion Chromatograms (MRM Mode) of Prednisolone Acetate in One Month 40°C Stability Sample in the Presence of PVP-I

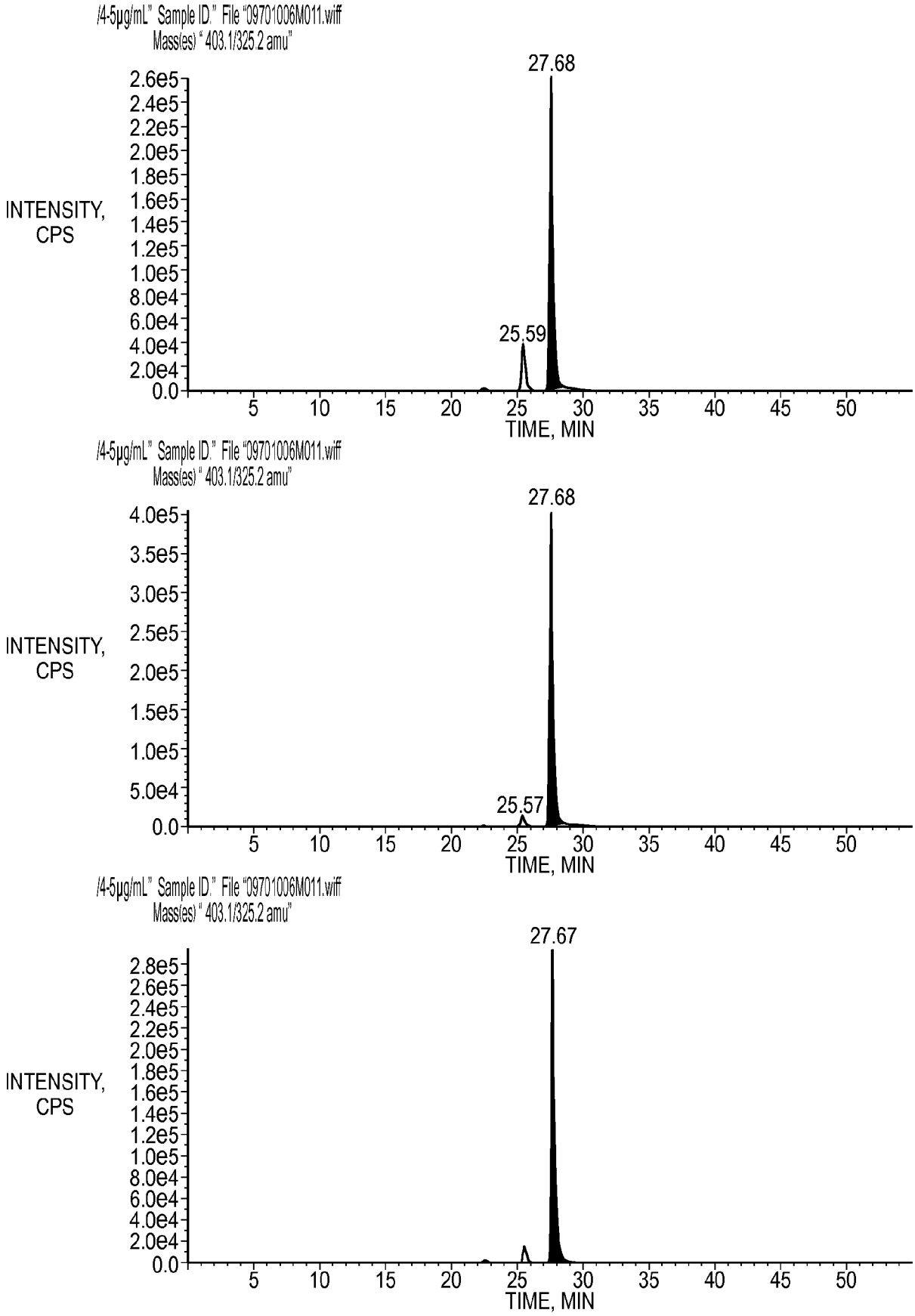

FIG. 26

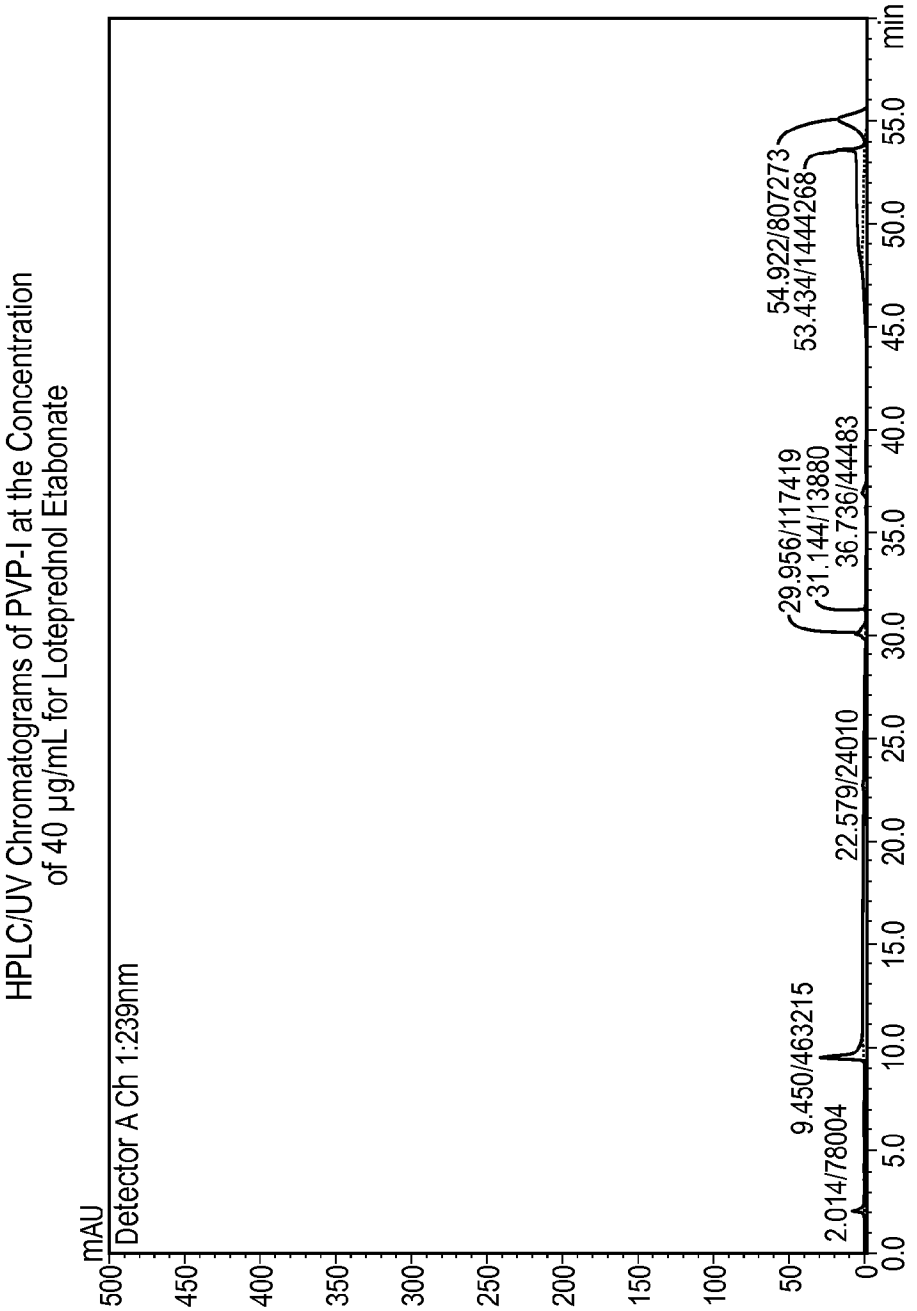
HPLC/UV Chromatograms of PVP-I at the Concentration of 40 µg/mL for Loteprednol Etabonate
Detector A Ch 1:239nm
mAU
500
450
400
350
300
250
200
150
100
50
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
min
2.014/78004
9.450/463215
22.579/24010
29.956/117419
31.144/13880
36.736/44483
53.434/1444268
54.922/807273

FIG. 27

HPLC/UV Chromatograms of Loteprednol Etabonate in PVP-I for Day 0

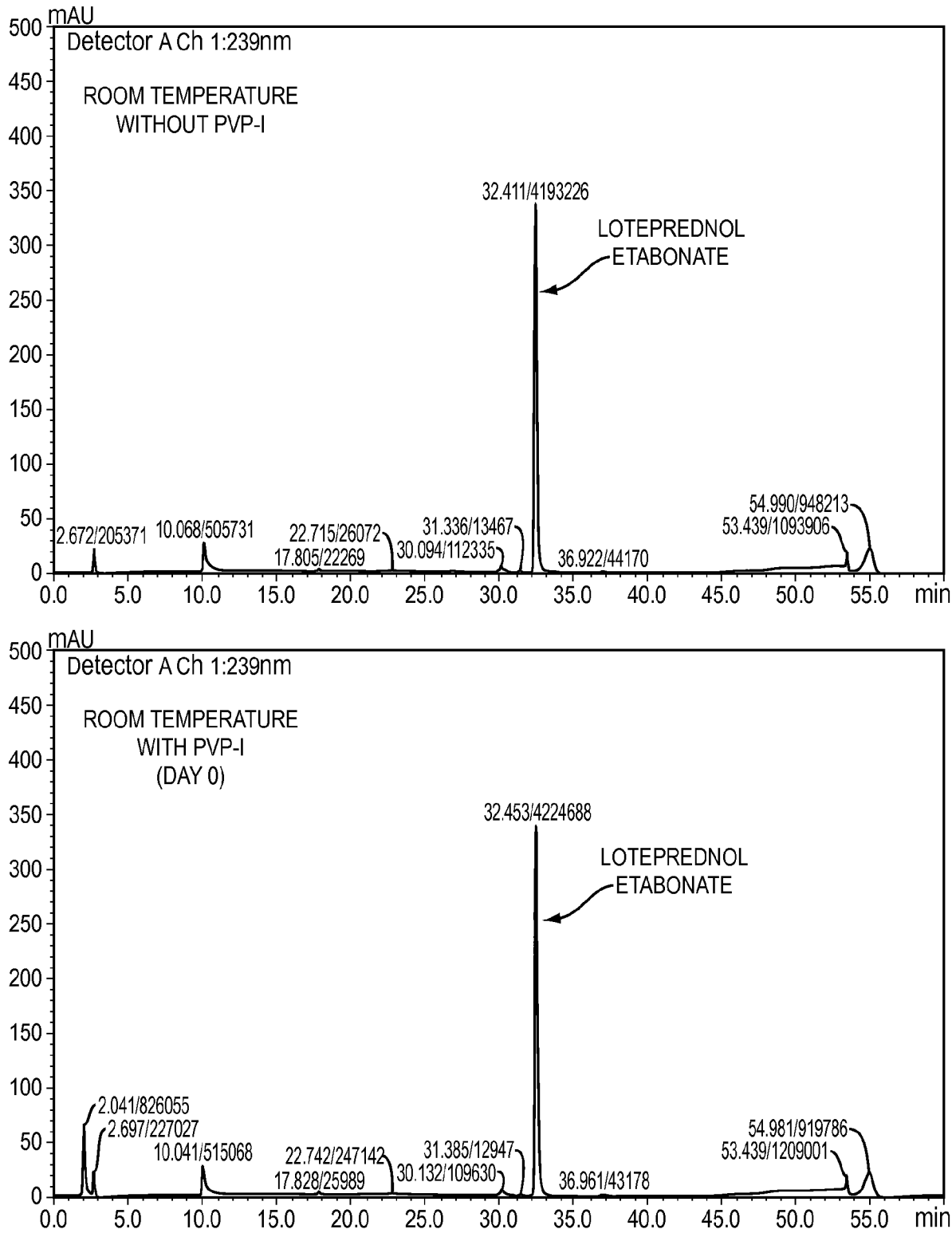

FIG. 28

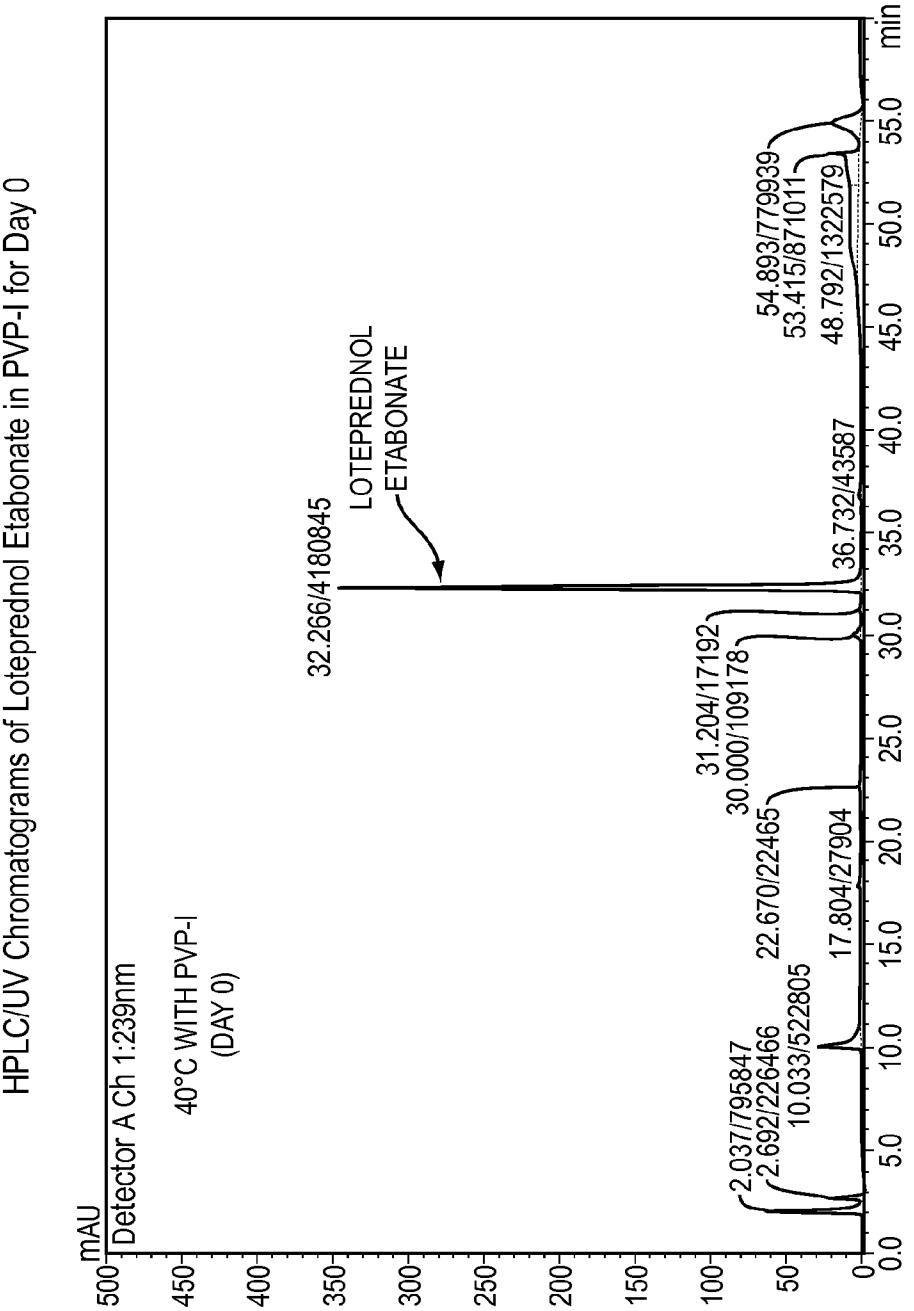
HPLC/UV Chromatograms of Loteprednol Etabonate in PVP-I for Day 0
Detector A Ch 1:239nm
40°C WITH PVP-I
(DAY 0)
mAU
min
LOTEPREDNOL
ETABONATE
32.266/4180845
2.037/795847
2.692/226466
10.033/522805
17.804/27904
22.670/22465
31.204/17192
30.000/109178
36.732/43587
48.792/1322579
53.415/871011
54.893/779939

FIG. 29

HPLC/UV Chromatograms of Loteprednol Etabonate in PVP-I for Two Weeks

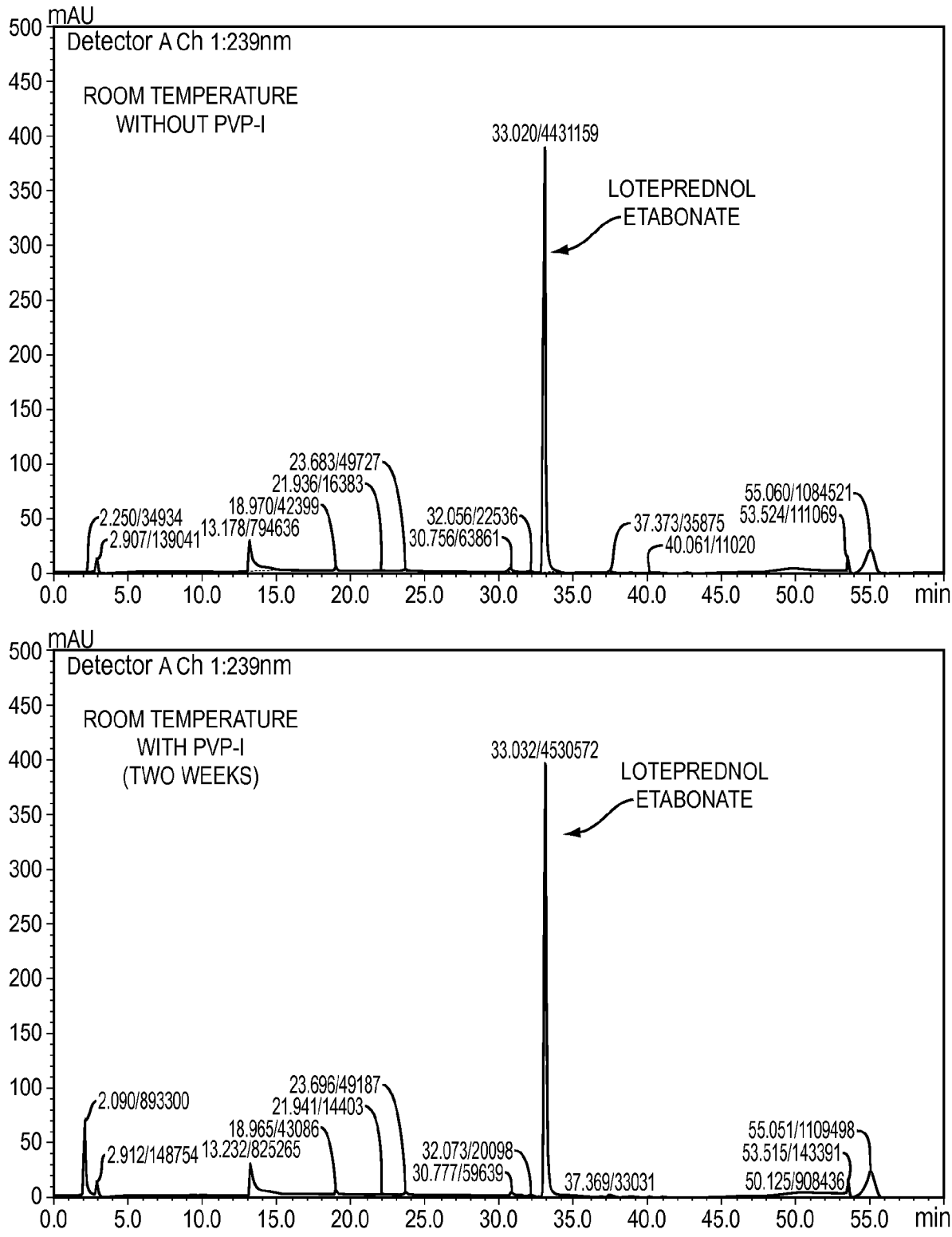

FIG. 30

HPLC/UV Chromatograms of Loteprednol Etabonate in PVP-I for Two Weeks

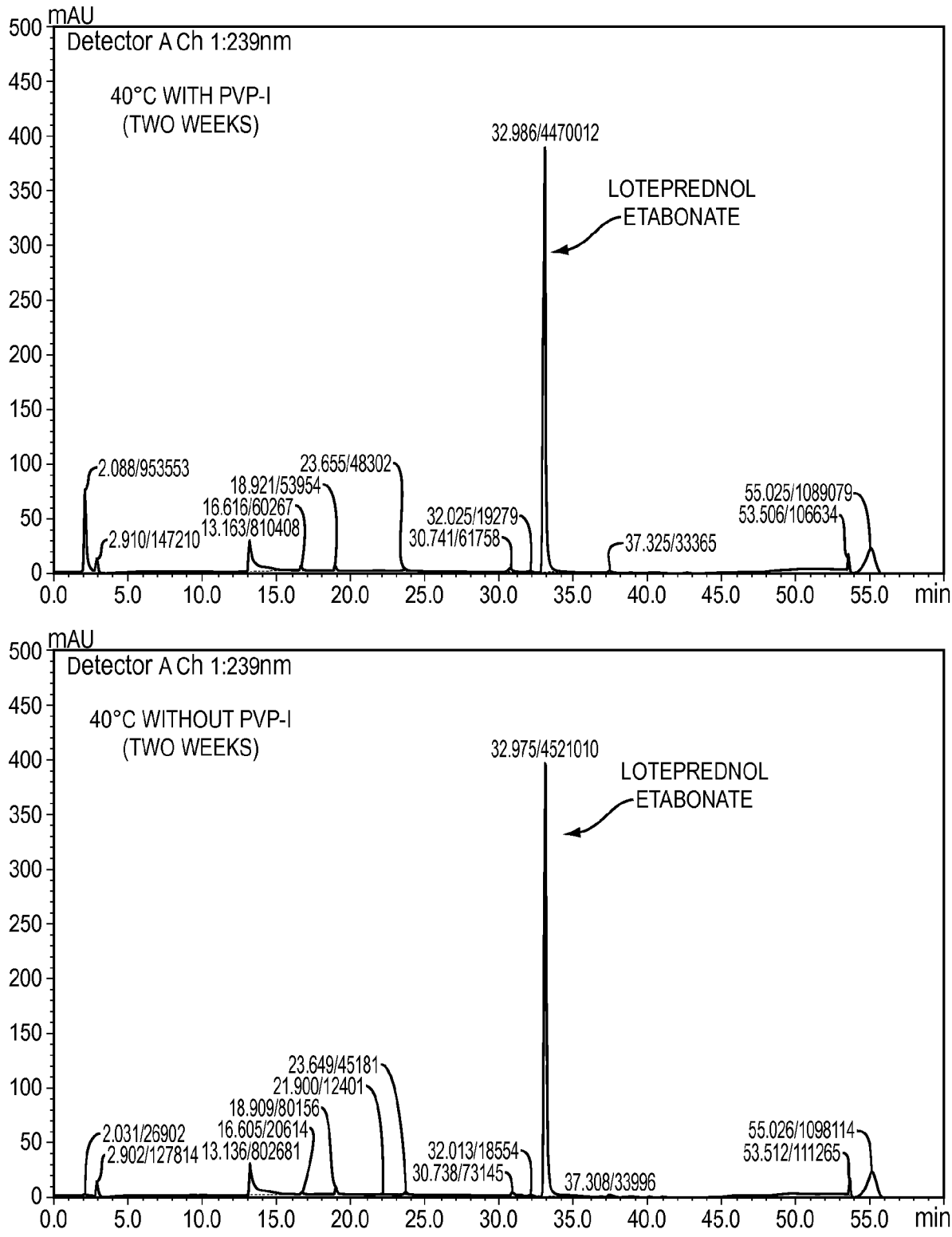

FIG. 31

HPLC/UV Chromatograms of Loteprednol Etabonate in PVP-I for One Month

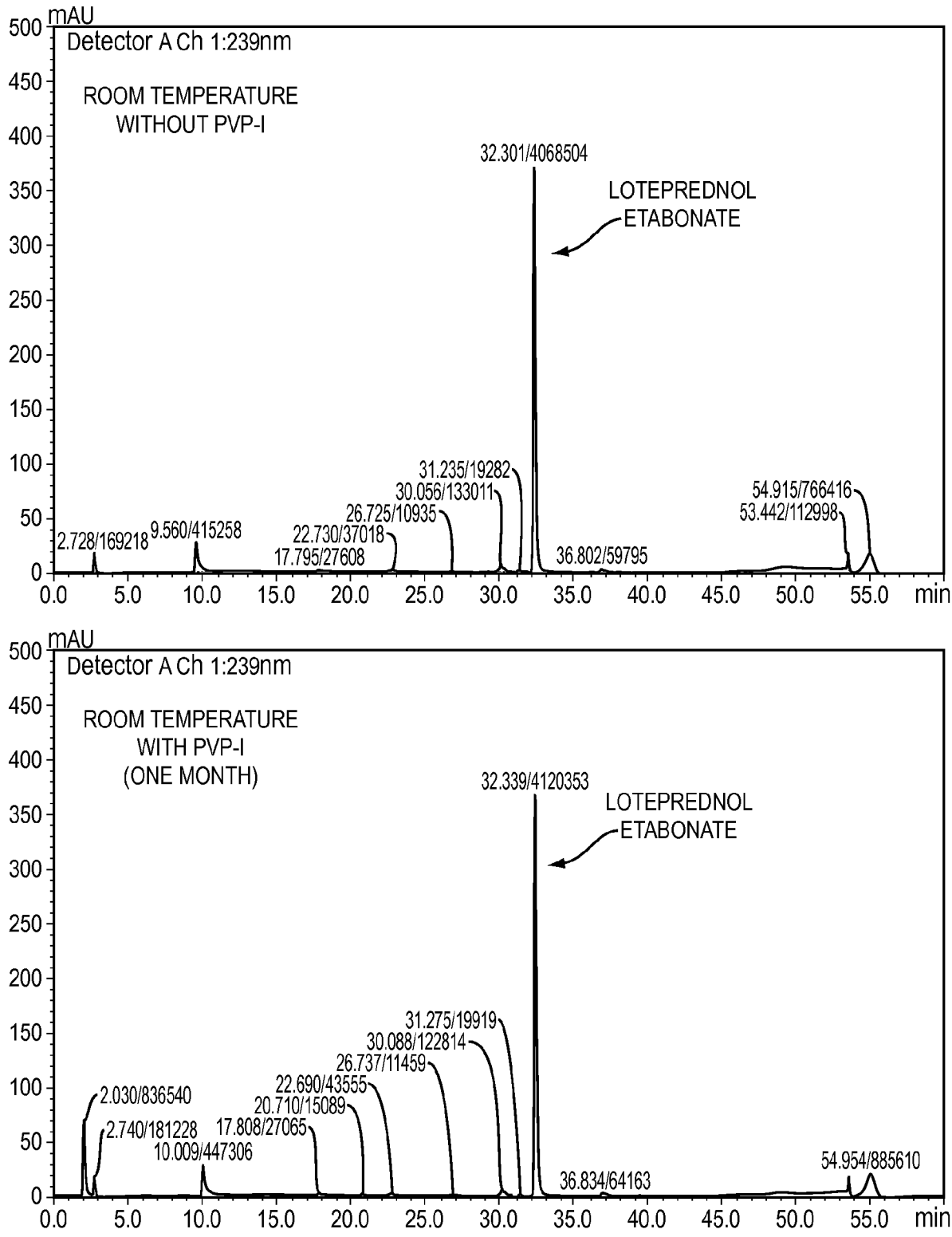

FIG. 32

HPLC/UV Chromatograms of Loteprednol Etabonate in PVP-I for One Month

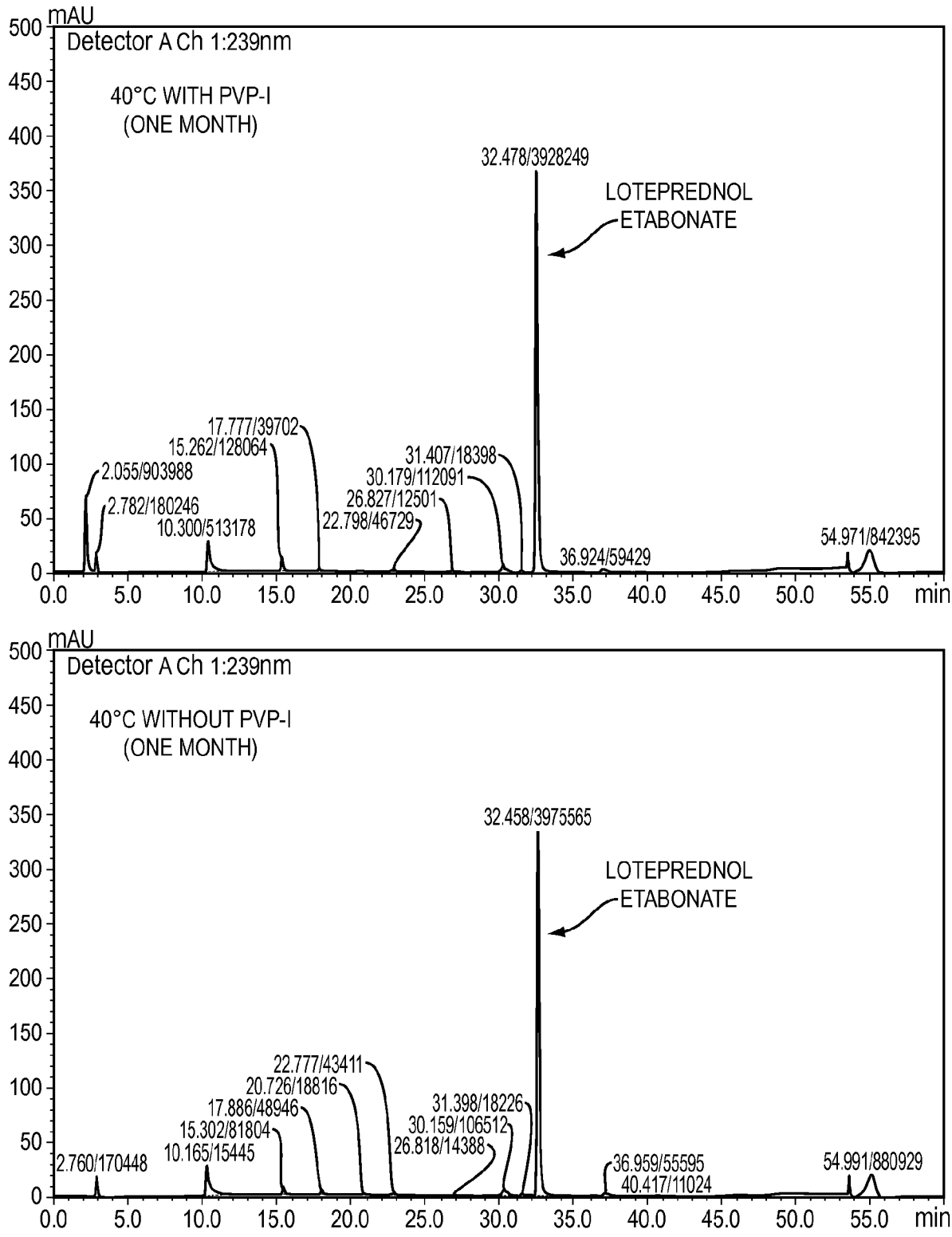

FIG. 33

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Mass Ion Chromatograms (MRM Mode) of Loteprednol Etabonate in Reference Standard Samples

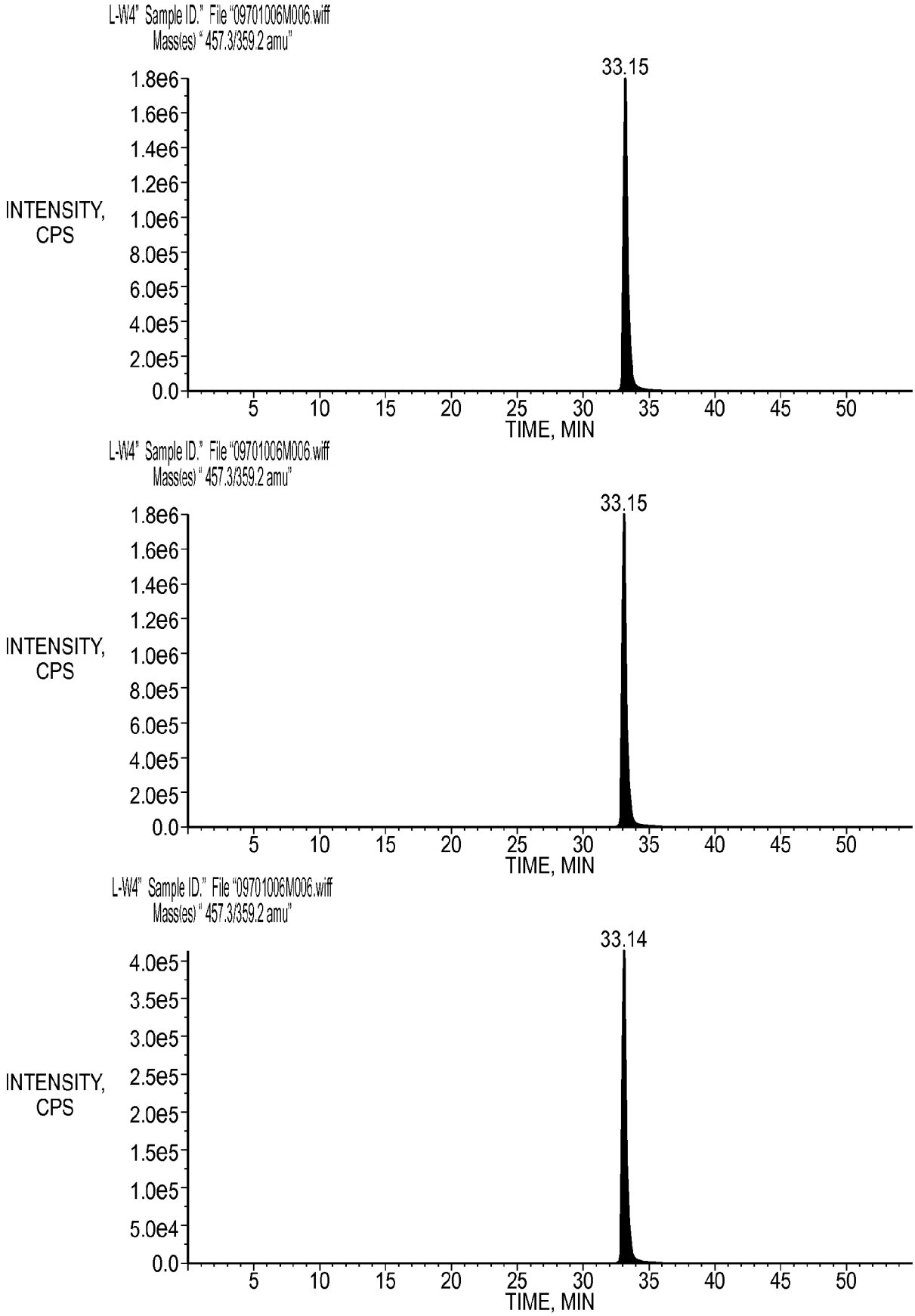

FIG. 34

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Mass Ion Chromatograms (MRM Mode) of Loteprednol Etabonate in One Month Room Temperature Stability Sample in the Presence of PVP-I

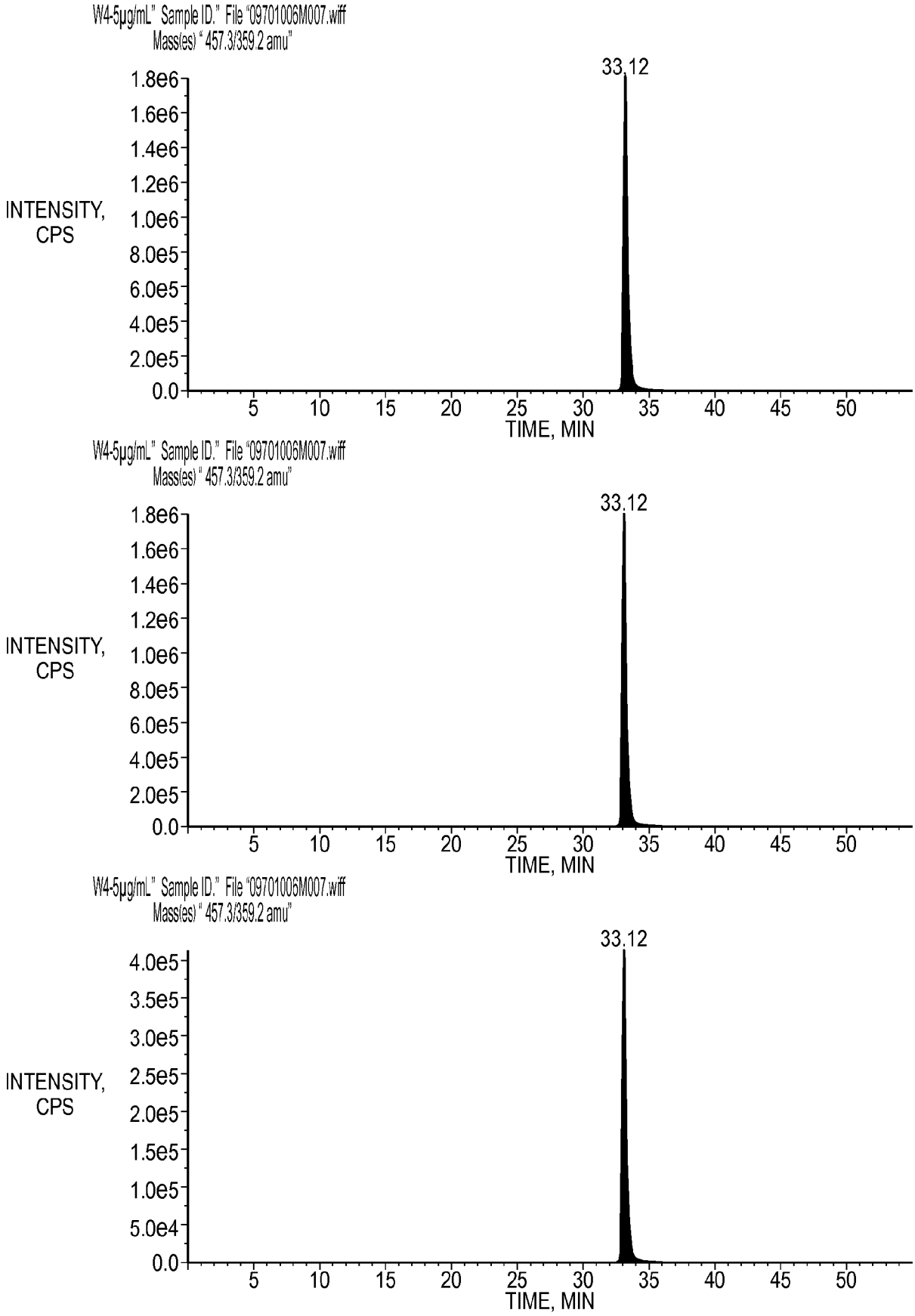

FIG. 35

Mass Ion Chromatograms (MRM Mode) of Loteprednol Etabonate in One Month 40°C Stability Sample in the Presence of PVP-I

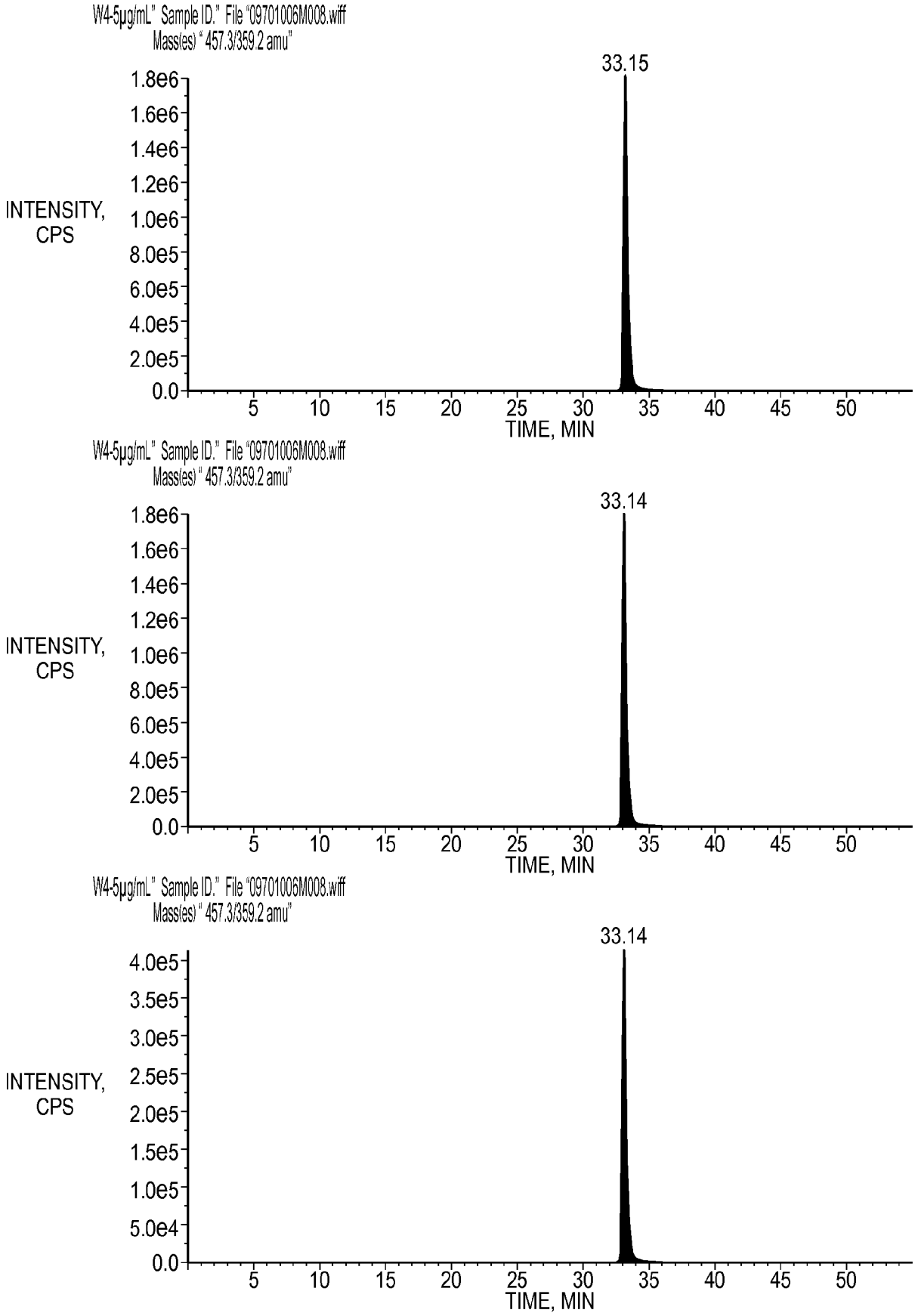

FIG. 36

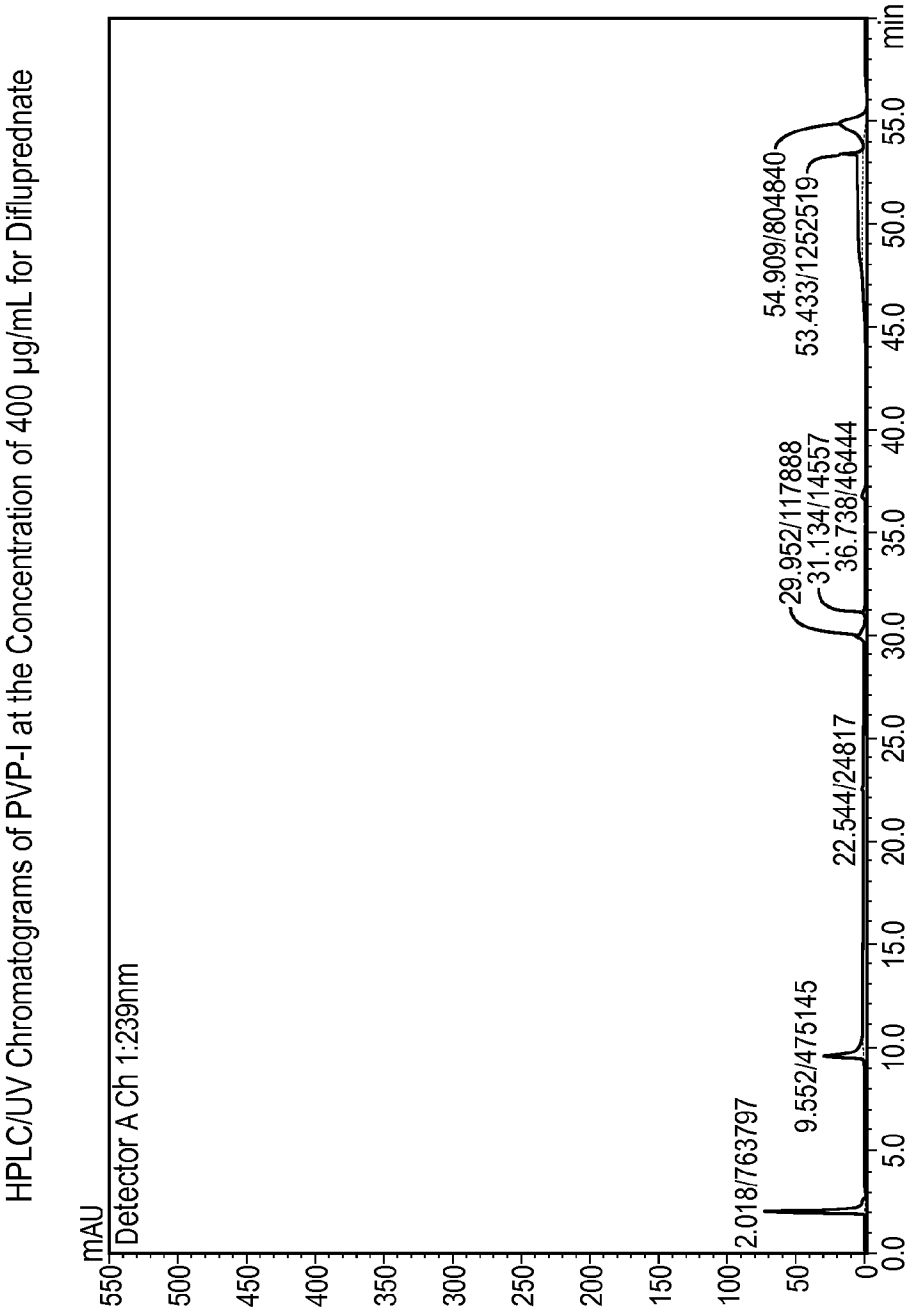
HPLC/UV Chromatograms of PVP-I at the Concentration of 400 µg/mL for Difluprednate
mAU
Detector A Ch 1:239nm
550
500
450
400
350
300
250
200
150
100
50
0
2.018/763797
9.552/475145
22.544/24817
29.952/117888
31.134/14557
36.738/46444
53.433/1252519
54.909/804840
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
min

FIG. 37

HPLC/UV Chromatograms of Difluprednate in PVP-I for Day 0

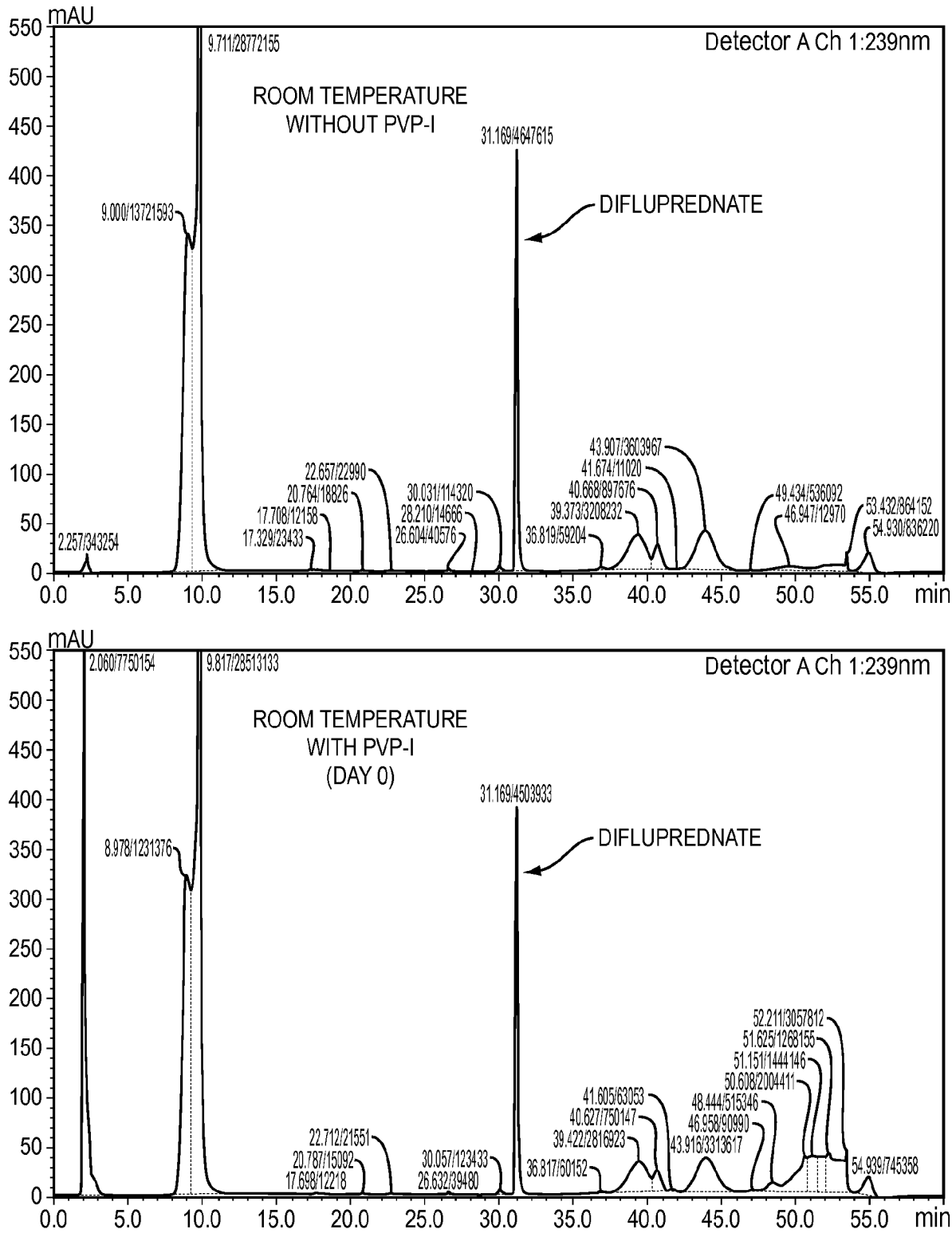

FIG. 38

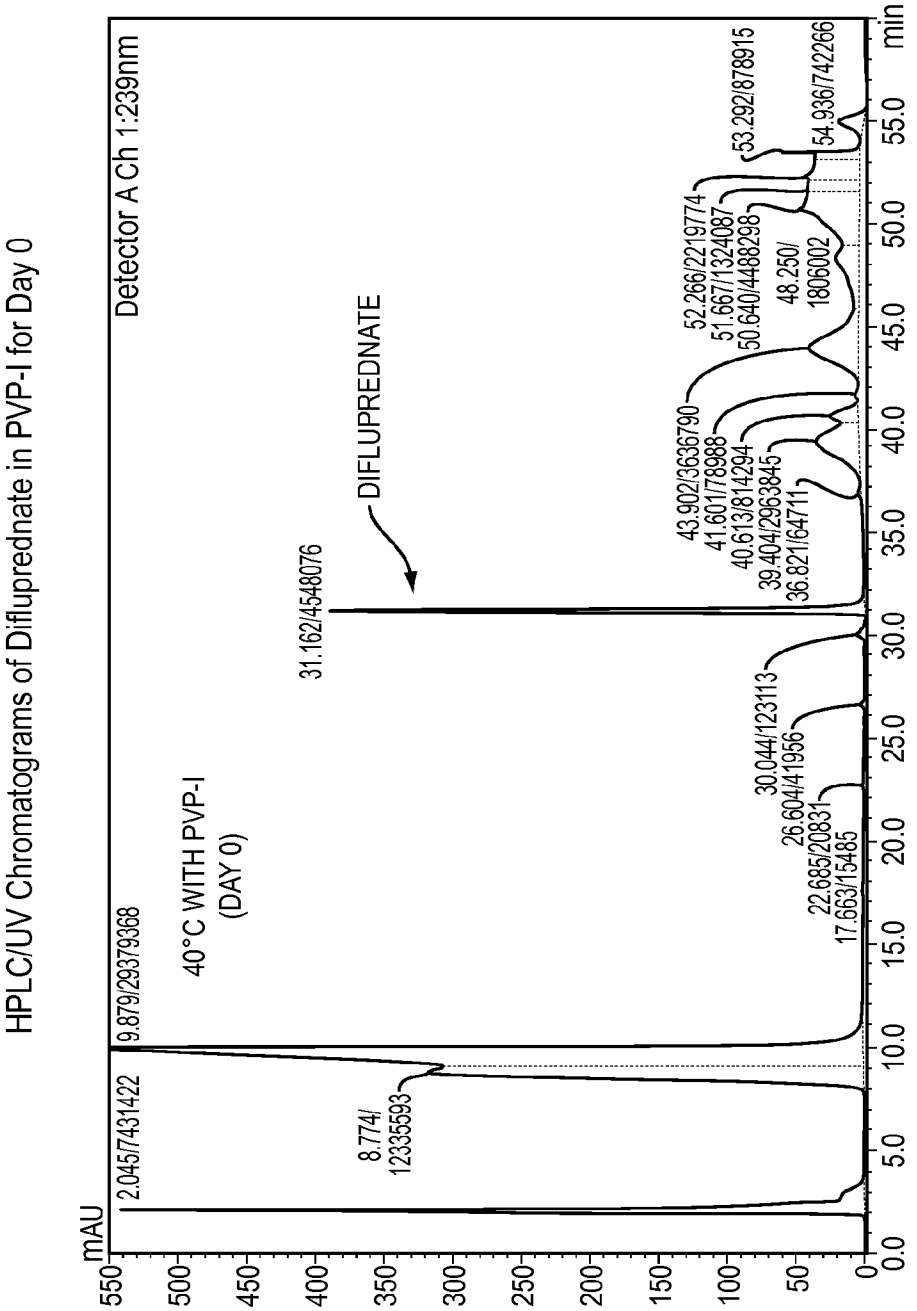
HPLC/UV Chromatograms of Difluprednate in PVP-I for Day 0
Detector A Ch 1:239nm
40°C WITH PVP-I
(DAY 0)
DIFLUPREDNATE
mAU
min
2.045/7431422
9.879/29379368
8.774/
12335593
31.162/4548076
30.044/123113
26.604/41956
22.685/20831
17.663/15485
43.902/3636790
41.601/78988
40.613/814294
39.404/2963845
36.821/64711
52.266/2219774
51.667/1324087
50.640/4488298
48.250/
1806002
53.292/878915
54.936/742266

FIG. 39

HPLC/UV Chromatograms of Difluprednate in PVP-I for Two Weeks

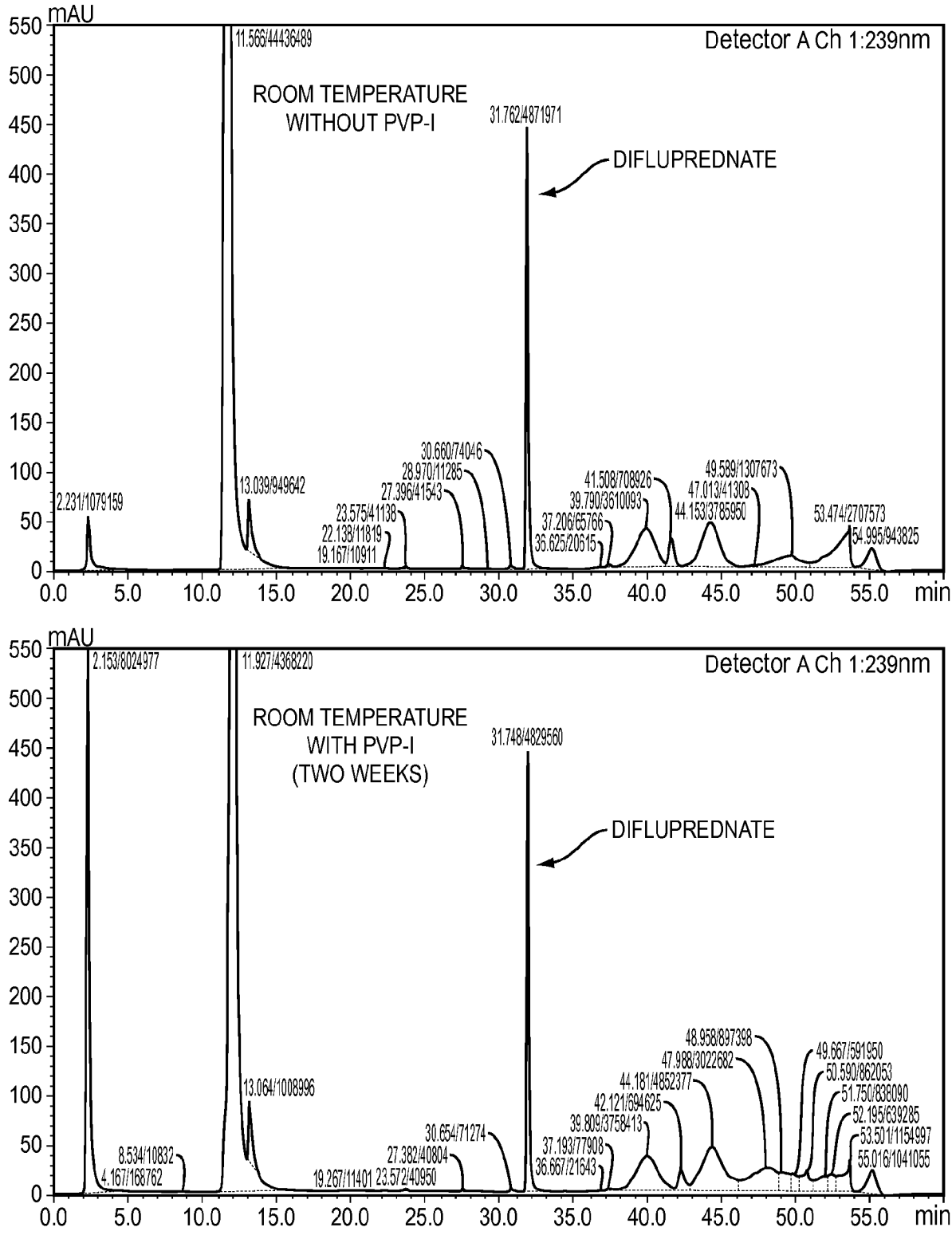

FIG. 40

HPLC/UV Chromatograms of Difluprednate in PVP-I for Two Weeks

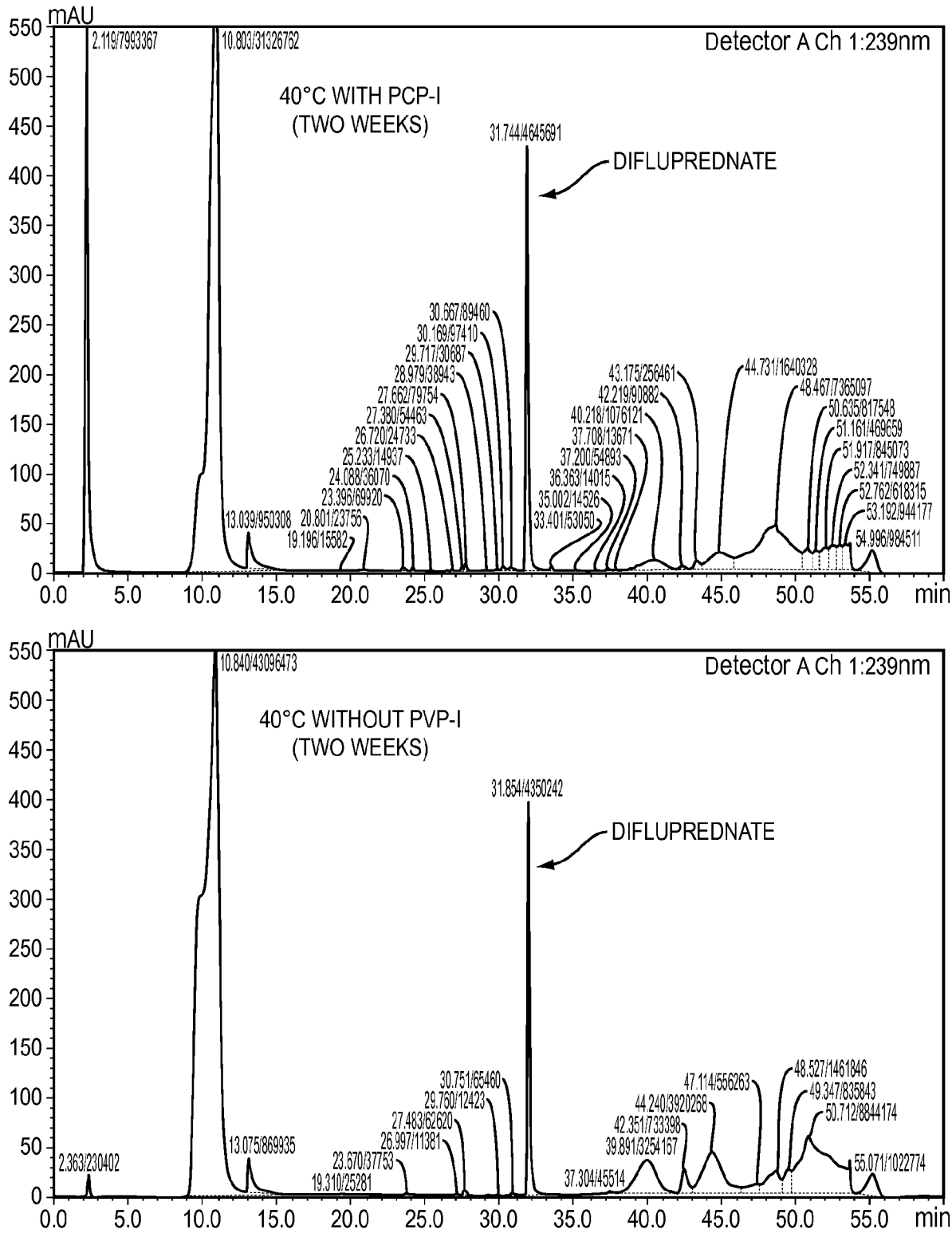

FIG. 41

HPLC/UV Chromatograms of Difluprednate in PVP-I for One Month

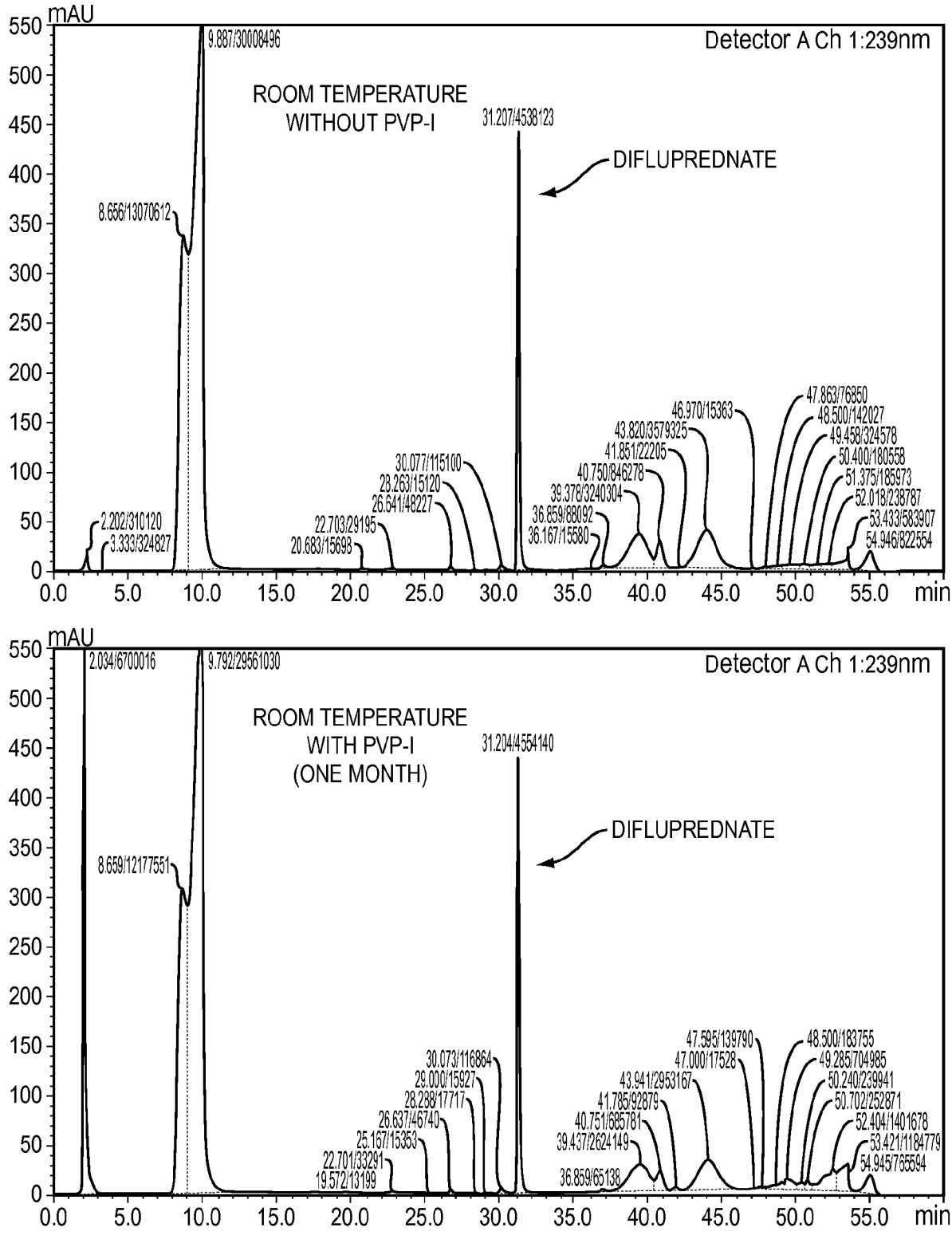

FIG. 42

HPLC/UV Chromatograms of Difluprednate in PVP-I for One Month

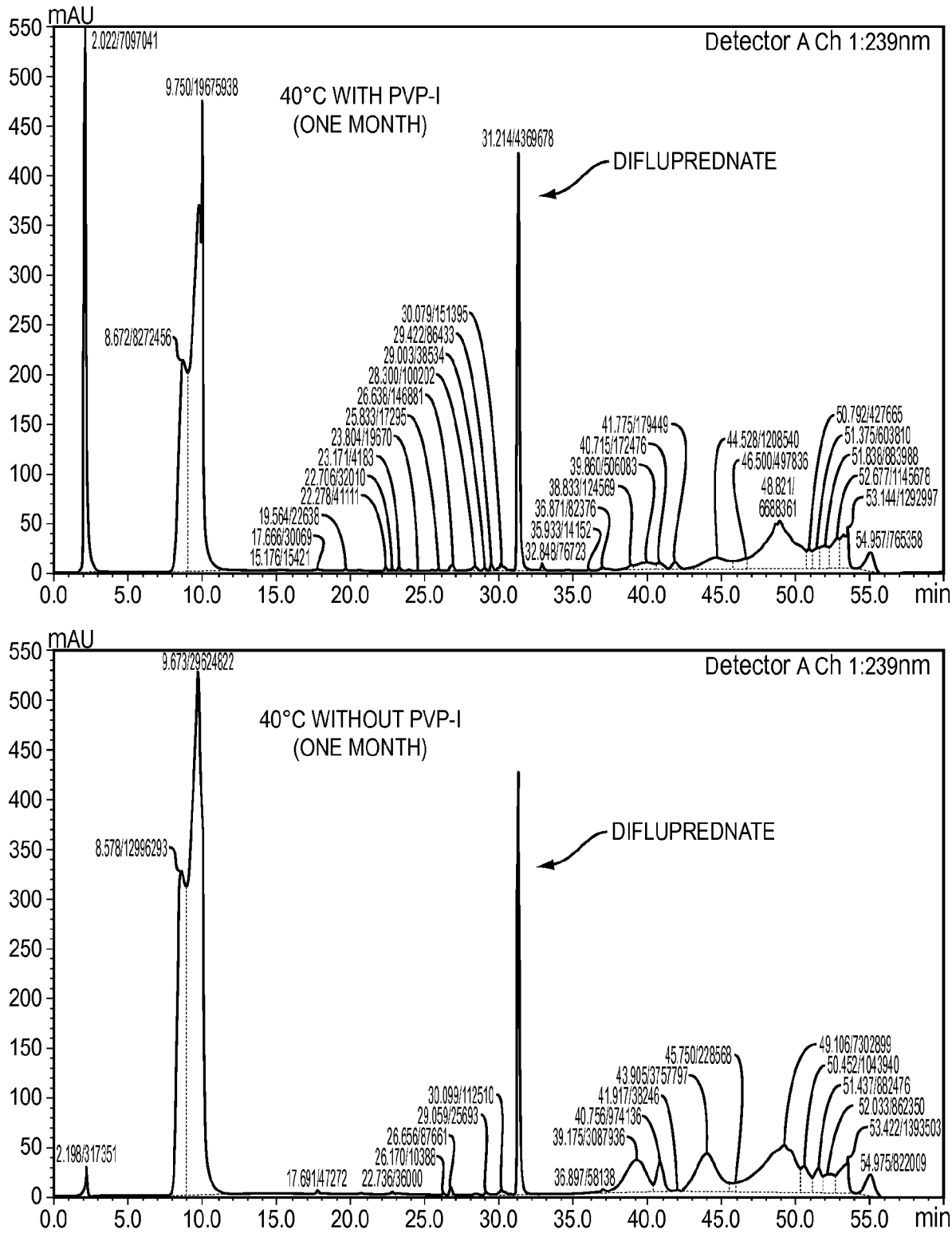

FIG. 43

Mass Ion Chromatograms (MRM Mode) of Difluprednate in Reference Standard Samples

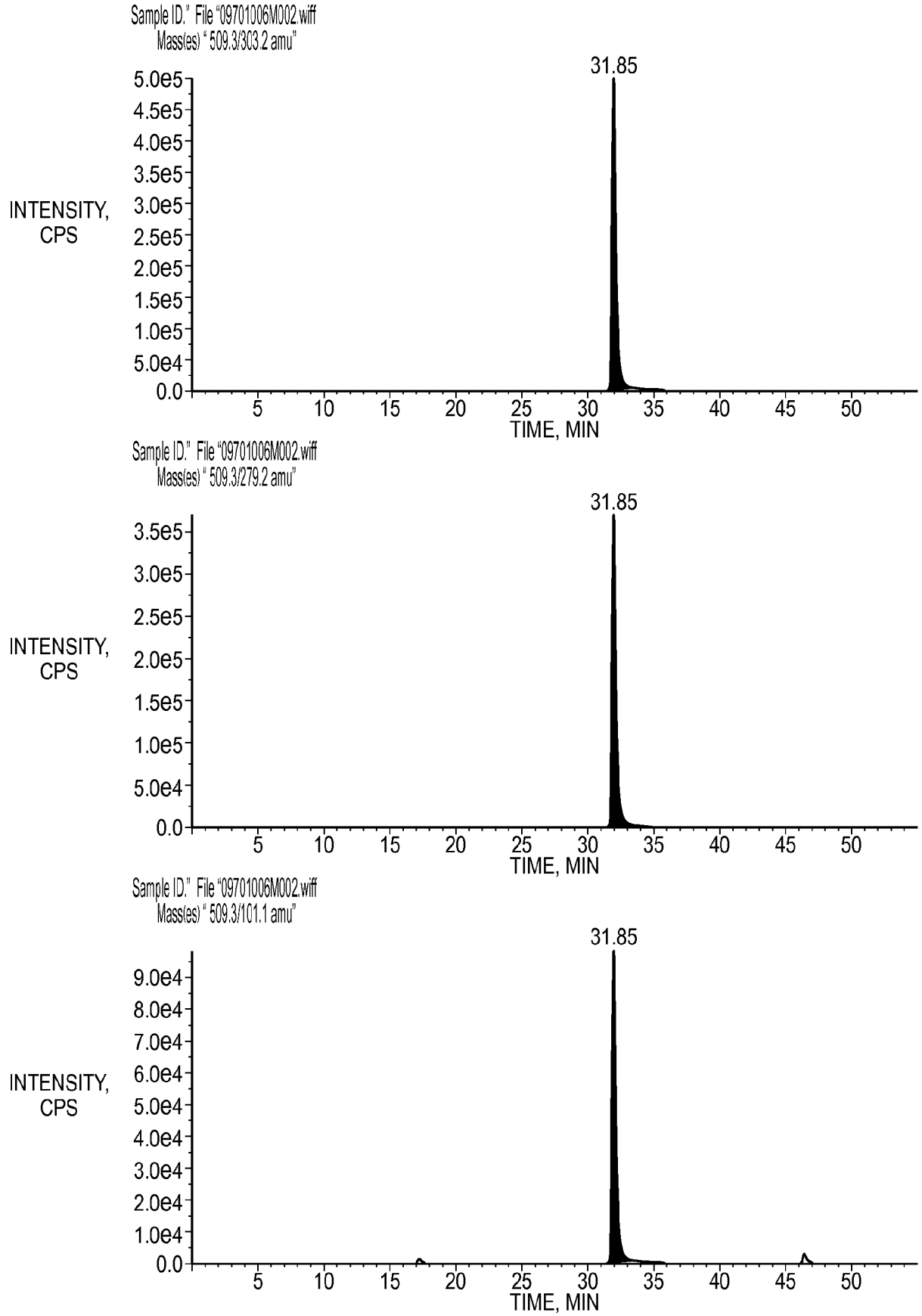

FIG. 44

Mass Ion Chromatograms (MRM Mode) of Difluprednate in One Month Room Temperature Stability Sample in the Presence of PVP-I

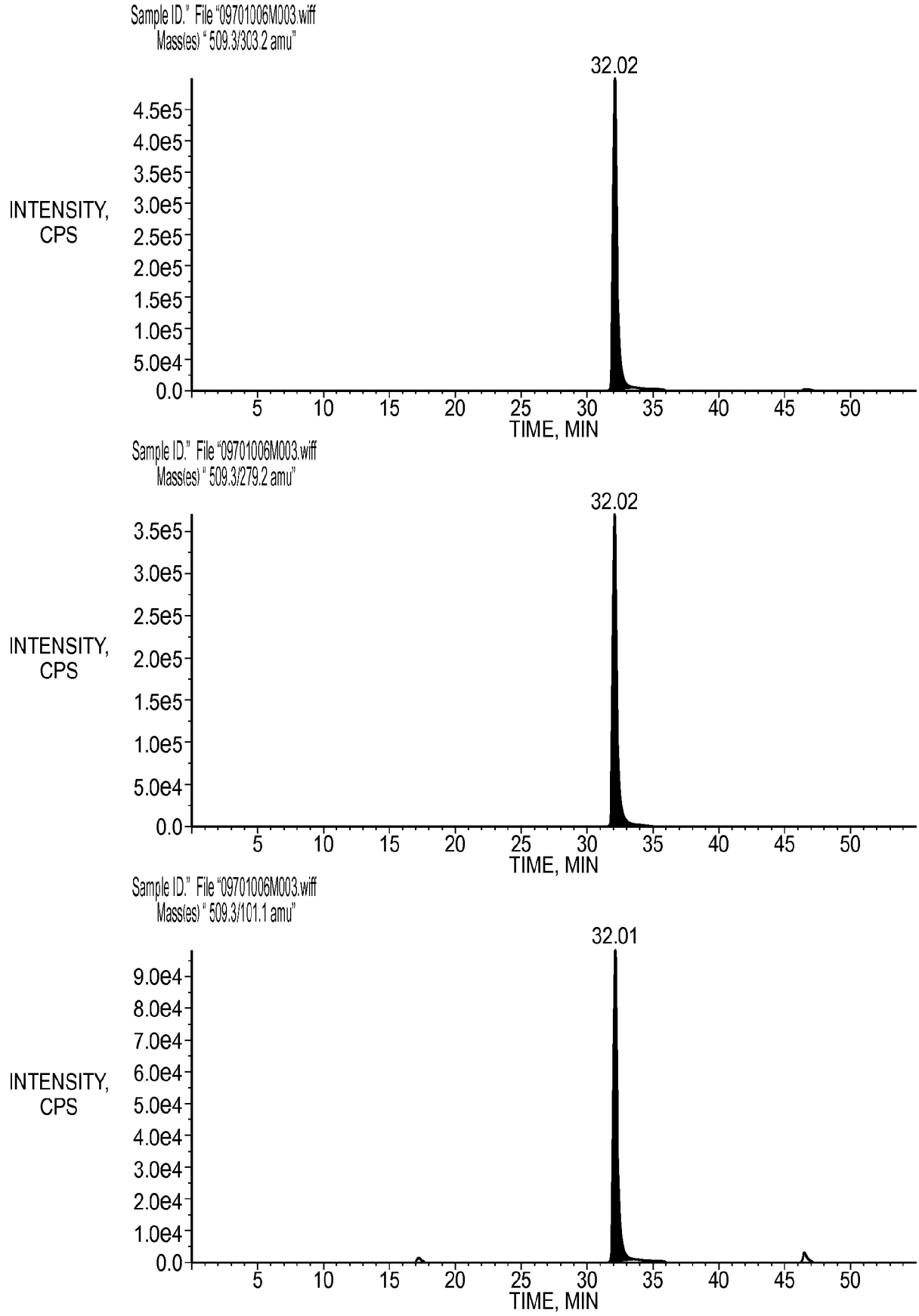

FIG. 45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/37563

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/74 (2012.01)
USPC - 424/78.04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 424/78.04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/667; 514/26, 169 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PUBWEST, Google, Google Scholar, WIPO, Opthalmic, eye, ocular, optic, topical, composition, formulation, method, treating, prophylaxis, ameliorate, administering, eye disorder, disease, infection, inflammation, conjunctivitis, corneal abrasion, ulcerative infectious keratitis, keratitis, bacteria, virus, fungi, amoebae, povidone, steroid, prednison

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7,767,217 B2 (Samson et al.) 03 August 2010 (03.08.2010) col 2, ln 3-12, ln 26-32, ln 62-66; col 3, ln 1-15, ln 23-26, ln 29-48, ln 59; col 4, ln 10-30; col 5, ln 13, ln 63-64; col 6, ln 15-18, ln 25-30; col 7, ln 63-65; col 8, ln 1-2; col 9, ln 1-7, ln 25-30, Example 1; col 11, ln 63-65	1-44
X	WO 2009/151619 A1 (Capriotti et al.) 17 December 2009 (17.12.2009) pg 1, ln 29-31; pg 2, ln 6; pg 3, ln 25-30; pg 4, ln 15, ln 20-21; pg 7, ln 25-31	45-46
X	US 2010/0254934 A1 (Samson et al.) 07 October 2010 (07.10.2010) abstract; para [0007]-[0009], [0011]-[0020], [0022], [0048]-[0052], [0071]-[0075]	1-44
X	US 2010/0291019 A1 (Samson et al.) 18 November 2010 (18.11.2010) abstract; para [0007]-[0009], [0011]-[0018], [0020]-[0022], [0047]-[0049], [0062]-[0069]	1-44
X	WO 2007/106381 A2 (Samson et al.) 20 September 2007 (20.09.2007) abstract; pg 2, para 3; pg 3, para 1, 4; pg 4, para 1-5; pg 11, para 1; pg 13, para 3-4; pg 14, para 1-4; pg14-16	1-44
X/P	US 2012/0027716 A1 (Stein et al.) 02 February 2012 (02.02.2012) entire document	1-46

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2012 (20.07.2012)	03 AUG 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)