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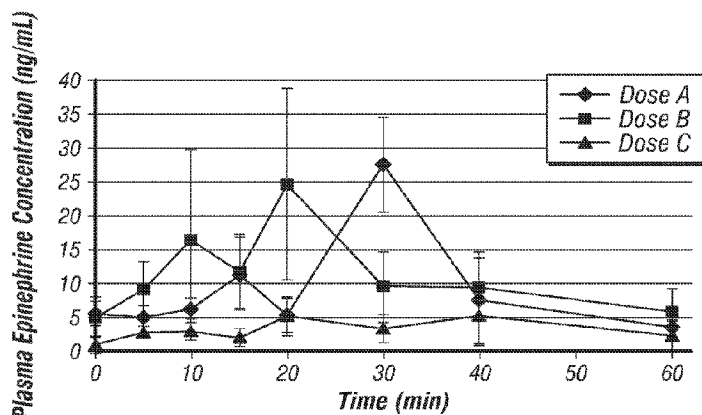
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(54) Title: EPINEPHRINE NANOPARTICLES, METHODS OF FABRICATION THEREOF, AND METHODS FOR USE
THEREOF FOR TREATMENT OF CONDITIONS RESPONSIVE TO EPINEPHRINE



(57) Abrégé/Abstract:

The invention provides compositions including epinephrine nanoparticles and methods for therapeutic use of the compositions for the treatment of conditions responsive to epinephrine such as a cardiac event or an allergic reaction, particularly anaphylaxis. The epinephrine nanoparticles can be incorporated into orally-disintegrating and fast-disintegrating tablet pharmaceutical formulations and can significantly increase the sublingual bioavailability of epinephrine, and thereby reduce the epinephrine dose required. Additionally, the invention provides methods for fabrication of stabilized epinephrine nanoparticles for use in the described compositions.

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(54) Title: EPINEPHRINE NANOPARTICLES, METHODS OF FABRICATION THEREOF, AND METHODS FOR USE THEREOF FOR TREATMENT OF CONDITIONS RESPONSIVE TO EPINEPHRINE

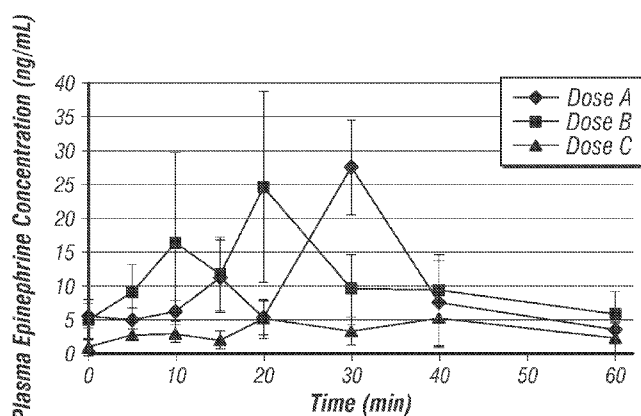


FIG. 1

(57) Abstract: The invention provides compositions including epinephrine nanoparticles and methods for therapeutic use of the compositions for the treatment of conditions responsive to epinephrine such as a cardiac event or an allergic reaction, particularly anaphylaxis. The epinephrine nanoparticles can be incorporated into orally-disintegrating and fast-disintegrating tablet pharmaceutical formulations and can significantly increase the sublingual bioavailability of epinephrine, and thereby reduce the epinephrine dose required. Additionally, the invention provides methods for fabrication of stabilized epinephrine nanoparticles for use in the described compositions.

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EPINEPHRINE NANOPARTICLES, METHODS OF FABRICATION
THEREOF, AND METHODS FOR USE THEREOF FOR TREATMENT OF
CONDITIONS RESPONSIVE TO EPINEPHRINE

5

FIELD OF THE INVENTION

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The invention generally relates to compositions and methods for
treatment of conditions responsive to epinephrine (also known as adrenaline),
particularly to compositions and methods for emergency treatment of conditions
responsive to epinephrine, and most particularly to compositions including
epinephrine nanoparticles for sublingual administration in treatment of

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conditions responsive to epinephrine.

BACKGROUND

5 Tablets that disintegrate or dissolve rapidly in the patient's mouth
without the use of water are convenient for the elderly, young children,
patients with swallowing difficulties, and in situations where water is not
available. For these specially designed formulations, the small volume of
saliva that is available is sufficient to disintegrate or dissolve a tablet in the
oral cavity. The drug released from these tablets can be absorbed partially or
10 entirely into the systemic circulation from the buccal mucosa or sublingual
cavity, or can be swallowed as a solution to be absorbed from the
gastrointestinal tract.

 The sublingual route usually produces a faster onset of action than
15 traditional orally-administered tablets and the portion absorbed through the
sublingual blood

vessels bypasses the hepatic first pass metabolic processes (Birudaraj et al., 2004, *J Pharm Sci* 94; Motwani et al., 1991, *Clin Pharmacokinet* 21: 83-94; Ishikawa et al., 2001, *Chem Pharm Bull* 49: 230-232; Price et al., 1997, *Obstet Gynecol* 89: 340-345; Kroboth et al., 1995, *J Clin Psychopharmacol* 15: 259-262; Cunningham et al., 1994, 5 *J Clin Anesth* 6: 430-433; Scavone et al., 1992, *Eur J Clin Pharmacol* 42: 439-443; Spenard et al., 1988, *Biopharm Drug Dispos* 9: 457-464).

Likewise, due to high buccal and sublingual vascularity, buccally- or sublingually-delivered drugs can gain direct access to the systemic circulation and are not subject to first-pass hepatic metabolism. In addition, therapeutic agents 10 administered via the buccal or sublingual route are not exposed to the acidic environment of the gastrointestinal tract (Mitra et al., 2002, *Encyclopedia of Pharm. Tech.*, 2081-2095). Further, the buccal and sublingual mucosas have low enzymatic activity relative to the nasal and rectal routes. Thus, the potential for drug inactivation due to biochemical degradation is less rapid and extensive than other administration 15 routes (de Varies et al., 1991, *Crit. Rev. Ther. Drug Carr. Syst.* 8: 271-303).

The buccal and sublingual mucosas are also highly accessible, which allows for the use of tablets which are painless, easily administered, easily removed, and easily targeted. Because the oral cavity consists of a pair of buccal mucosa, tablets, such as fast disintegrating tablets, can be applied at various sites either on the same 20 mucosa or, alternatively, on the left or right buccal mucosa (Mitra et al., 2002, *Encyclopedia of Pharm. Tech.*, 2081-2095). In addition, the buccal and sublingual routes could be useful for drug administration to unconscious patients, patients undergoing an anaphylactic attack, or patients who sense the onset of an anaphylactic attack.

Anaphylaxis is a sudden, severe systemic allergic reaction, which can be fatal within minutes. Epinephrine (Epi) is the drug of choice for the treatment of anaphylaxis worldwide (Joint Task Force on Practice Parameters, 2005, *J Allergy Clin Immunol* 115: S483-S523; Lieberman, 2003, *Curr Opin Allergy Clin Immunol* 3: 313-318; Simons, 2004, *J Allergy Clin Immunol* 113: 837-844). It is available as an injectable dosage form in ampoules or in autoinjectors, however these are underused when anaphylaxis occurs (Simons, F.E.R. *J Allergy Clin Immunol* 124(4):625-636 2009; Simons, F.E.R. *J Allergy Clin Immunol* 125:S161-181 2010). The drawbacks of Epi auto-injectors include high cost, perceived large size and bulkiness, limitations on repeated dosing (if required), fear and anxiety associated with the use of needles (especially in children), and dosing errors caused by incorrect techniques of administration (Simons, K.J. et al. *Current Opinion in Clinical Immunology* 10:354-361 2010). Furthermore, in aqueous solutions, epinephrine is unstable in the presence of light, oxygen, heat, and neutral or alkaline pH values (Connors et al., 1986, in Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists, Wiley-Interscience Publication: New York) and thus has limited shelf-life; approximately one year.

The sublingual route of administration is a promising alternative route for epinephrine administration. The formulation of sublingual tablets of epinephrine would enable the development of tablets with a range of epinephrine doses to match the population on an mg/kg basis. Sublingual tablets of epinephrine would be easy to carry and self-administer eliminating the fear and anxiety associated with needles used in autoinjectors for young children, as well as readily providing the capability of multiple doses. Feasibility studies in humans and animals have shown that epinephrine can be absorbed sublingually (Gu et al., 2002, *Biopharm Drug Dispos* 23:

213-216; Simons et al., 2004, *J Allergy Clin Immunol* 113: 425-438). The recommended dose of epinephrine for the treatment of anaphylaxis is about 0.01 mg/Kg: usually about 0.2 mL to about 0.5 mL of a 1:1000 dilution of epinephrine in a suitable carrier. Based on historical and anecdotal evidence, an approximately 0.3 mg
5 dose of epinephrine, by subcutaneous (SC) or intramuscular (IM) injection into the deltoid muscle, has been agreed upon as the dose required for the emergency treatment of anaphylaxis. Recent studies have demonstrated that if the approximately 0.3 mg dose is administered IM into the laterus vascularis (thigh) muscle, Epi plasma concentrations are higher and occur more quickly than SC or IM administration into
10 the deltoid muscle. (Joint Task Force on Practice Parameters, 2005, *J Allergy Clin Immunol* 115: S483-S523; Lieberman, 2003, *Curr Opin Allergy Clin Immunol* 3: 313-318; Simons, 2004, *J Allergy Clin Immunol* 113: 837-844)).

As stated above, epinephrine (Epi) is typically administered either subcutaneously (SC) or intramuscularly (IM) by injection. Thus, Epi injections are the
15 accepted first aid means of delivering Epi and are administered either manually or by automatic injectors. It is recommended that persons at risk of anaphylaxis, and persons responsible for children at risk for anaphylaxis, maintain one or more automatic Epi injectors in a convenient place at all times.

Given the difficulties associated with manual subcutaneous (SC) or
20 intramuscular (IM) administration of Epi, such as patient apprehension related to injections or the burden of an at risk person having to always maintain an Epi injector close at hand, there exists a need in the art for more convenient dosage forms which can provide immediate administration of Epi, particularly to a person undergoing anaphylaxis wherein the need for injection or Epi injectors is obviated.

Recently, a novel fast-disintegrating tablet suitable for sublingual (SL) administration of Epi was developed. *See* related U.S. applications: U.S. Provisional Patent Application No. 60/715,180; U.S. Provisional Patent Application No. 60/759,039; U.S. Utility Patent Application No. 11/672,503; and U.S. Utility Patent Application No. 11/530,360. Sublingual administration of 40 mg epinephrine as the bitartrate salt using these novel tablets resulted in a rate and an extent of epinephrine absorption similar to that achieved following intramuscular injections of 0.3 mg epinephrine in the thigh. Sublingual doses ranging from 5 to 40 mg epinephrine as the bitartrate salt were studied to achieve equivalent plasma concentrations. In an animal model, it was determined that a 40 mg epinephrine dose administered sublingually as a bitartrate salt in tablet form resulted in plasma epinephrine concentrations similar to those achieved by 0.3 mg epinephrine intramuscular (IM) injection (Rawas-Qalaji et al. *J Allergy Clin Immunol* 117:398-403 2006).

Without being bound by theory, it is thought that fabrication of epinephrine into nanoparticles and incorporation of the nanoparticles into a tablet formulation with pharmaceutically-acceptable carriers, penetration enhancers, and mucoadhesives will significantly increase the absorption of SL-administered epinephrine and will result in the reduction of SL epinephrine dose required.

SUMMARY OF THE INVENTION

Epinephrine (Epi) is life-saving in the treatment of anaphylaxis. In community settings, a first-aid dose of epinephrine in an amount of 0.15 mg or 0.3 mg is injected into the mid-outer thigh by patients or caregivers using an auto-injector such as an EpiPen® (epinephrine auto-injector 0.3/0.15 mg, Dey Pharma, L.P. Nappa, CA). Epi auto-injectors are under-used because of needle phobia, bulky size, and high cost;

additionally, there are only two fixed doses, shelf-life is only 12-18 months, and unintentional injection and injury sometimes occur.

The instant invention circumvents the aforementioned problems by providing a fast-disintegrating epinephrine tablet formulation for anaphylaxis treatment.

5 Although this formulation was designed with regard to anaphylaxis, it is equally effective and contemplated for use in treatment of any condition responsive to epinephrine such as cardiac events, *i.e.* cardiac arrest, and breathing difficulties, *i.e.* asthma, bronchial asthma, bronchitis, emphysema, and respiratory infections.

In a validated rabbit model, this fast-disintegrating epinephrine tablet
10 formulation resulted in plasma epinephrine concentrations similar to those achieved after a 0.3 mg epinephrine intra-muscular injection (Rawas-Qalaji et al. *J Allergy Clin Immunol* 117:398-403 2006). Furthermore, epinephrine was stable in these fast-disintegrating tablets for at least seven years.

In one aspect, the invention provides epinephrine nanoparticles. The
15 epinephrine can be either an epinephrine base or an epinephrine bitartrate salt.

The invention also provides stabilized epinephrine nanoparticles.

In another aspect, the invention provides a composition including epinephrine nanoparticles capable of enhancing the sublingual bioavailability of epinephrine for the emergency treatment of anaphylaxis.

20 The invention additionally provides a method for fabrication of stabilized epinephrine nanoparticles and incorporation of the fabricated nanoparticles into orally-disintegrating and fast-disintegrating tablets. The fabrication method includes combining a pre-determined amount of epinephrine (epinephrine base or epinephrine bitartrate salt) and a solvent in a reaction chamber to form a mixture and exposing the
25 mixture to at least one pass at a pre-determined pressure and a pre-determined

temperature. The pre-determined pressure ranges from about 8,000 psi to 30,000 psi. The pre-determined temperature ranges from 8.3 to 43.3 °C. The solvent with which the epinephrine is combined can be water with or without sodium metabisulfite, isopropyl alcohol (ISP), methanol, acetonitrile, acetone, hexane, chloroform, dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate, phosphoric acid, and acetic acid.

As described herein, buccal or sublingual oral disintegrating tablets (ODTs) are distinguished from conventional sublingual tablets, lozenges, or buccal tablets by the ODTs' ability to fully dissolve or disintegrate in less than about one minute in the mouth.

The fabrication method for stabilized epinephrine nanoparticles can also include exposing the mixture of epinephrine and solvent to a second pass at a different pre-determined pressure and a different pre-determined temperature from that of the first pass.

Additionally, nanoparticles fabricated by the method can be lyophilized (freeze-dried).

In another aspect, the invention provides a pharmaceutical composition including epinephrine nanoparticles formulated for buccal or sublingual administration.

The invention also provides a pharmaceutical composition including epinephrine nanoparticles and a pharmaceutically-acceptable carrier for buccal or sublingual administration.

The phrase "pharmaceutically-acceptable carrier" refers to an inactive and non-toxic substance used in association with an active substance, *i.e.* epinephrine, especially for aiding in the application of the active substance. Non-limiting

examples of pharmaceutically-acceptable carriers are diluents, binders, disintegrants, flavorings, fillers, and lubricants. Pharmaceutically-acceptable carriers can have more than one function, *i.e.* a filler can also be a disintegrant. Additionally, pharmaceutically-acceptable carriers may also be referred to as non-medicinal
5 ingredients (NMIs).

The invention also provides a pharmaceutical composition, for buccal or sublingual administration, including epinephrine nanoparticles and at least one of a pharmaceutically-acceptable carrier, a penetration enhancer, and a mucoadhesive. The pharmaceutical composition can further include at least one of a taste enhancer
10 and a sweetening agent and mouthfeel enhancer. A non-limiting example of a taste enhancer is citric acid. Citric acid masks the bitter taste of epinephrine. A non-limiting example of a sweetening agent and mouthfeel enhancer is mannitol. The pharmaceutical composition can further include at least one of a filler, a lubricant, and a disintegrant. Non-limiting examples include microcrystalline cellulose (filler),
15 magnesium stearate (lubricant), and hydroxypropyl ethers of cellulose (disintegrant).

Additionally, the invention provides a pharmaceutical composition including epinephrine nanoparticles, in which the bitter taste of the epinephrine is masked by a taste enhancer. A non-limiting example of a taste enhancer is citric acid.

In another aspect, the invention provides a method for enhancing sublingual
20 bioavailability of epinephrine in a subject in need thereof including steps for providing a composition including epinephrine nanoparticles and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. The described fast-disintegrating epinephrine tablets enhance bioavailability of epinephrine by releasing epinephrine within sixty seconds of administration.

In another aspect, the invention provides a method for treating a condition responsive to epinephrine in a subject in need thereof including steps for providing a composition including epinephrine nanoparticles and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. Conditions responsive to epinephrine react to administration of epinephrine. Non-limiting examples of conditions responsive to epinephrine include a cardiac event, *i.e.* cardiac arrest, or an allergic reaction, *i.e.* anaphylaxis, asthma, or bronchial asthma.

The phrase “effective amount” refers to the amount of a composition necessary to achieve the composition’s intended function.

10 The phrase “therapeutically-effective amount” refers to the amount of a composition required to achieve the desired function, *i.e.* treatment of the condition responsive to epinephrine.

In another aspect, the invention provides a method for treating a breathing difficulty in a subject in need thereof including steps for providing a composition including epinephrine nanoparticles and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. Breathing difficulties responsive to epinephrine include, but are not limited to, breathing difficulties associated with anaphylaxis, asthma, bronchial asthma, bronchitis, emphysema, and respiratory infections.

20 The invention additionally provides a method for treatment of an allergic emergency in a subject diagnosed with or suspected of having an allergic emergency including steps for providing a composition including epinephrine nanoparticles and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. Non-limiting examples of allergic emergencies are anaphylaxis, asthma, and bronchial asthma.

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In an additional aspect, the invention provides a method for treatment of a cardiac event in a subject diagnosed with or suspected of having a cardiac event including steps for providing a composition including epinephrine nanoparticles and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. A non-limiting example of a cardiac event is cardiac arrest.

Also provided is a pharmaceutical composition formulated as a fast-disintegrating tablet, the pharmaceutical composition comprising:

a pharmaceutically effective dose of an active ingredient having about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size (Z-average) ranging from about 279 nm to about 196 nm; and
at least one non-medicinal ingredient (NMI).

Also provided is a pharmaceutical composition formulated as a fast-disintegrating tablet, the pharmaceutical composition comprising:

a pharmaceutically effective dose of an active ingredient having about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size measured as a Z-average of 230 ± 39 nm with a coefficient of variation (RSD) of 17%; and
at least one non-medicinal ingredient (NMI);

wherein the stabilized nanoparticles of epinephrine bitartrate are prepared from a mixture of epinephrine bitartrate and a solvent exposed to at least one pass at a pressure in a range of 8,000-30,000 psi and at a temperature in a range of 8.3 to 43.3 °C, wherein the solvent is at least one of water with or without sodium metabisulfite, isopropyl alcohol (ISP), methanol, acetonitrile, acetone, hexane, chloroform, dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate, phosphoric acid, and acetic acid.

Also provided is a pharmaceutically effective dose of about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size (Z-average) ranging from about 279 nm to about 196 nm, and at least one non-medicinal ingredient for use in increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine.

Also provided is a pharmaceutically effective dose of about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size (Z-average) ranging from about 279 nm to about 196 nm, and at least one non-medicinal ingredient for use in the manufacture of a composition for increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine.

Also provided is a pharmaceutical composition comprising at least an active ingredient having about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size of 230±39 nm measured as a Z-average according to ISO 13321.

Also provided is a pharmaceutical composition formulated as a fast-disintegrating tablet, the pharmaceutical composition comprising:

an active ingredient having about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size of 230±39 nm measured as a Z-average according to ISO 13321 and having a coefficient of variation (RSD) of 17% at a concentration of 2.8 mg/ml in isopropyl alcohol and at a pressure of 30,000 psi; and

a non-medicinal ingredient (NMI), wherein the non-medicinal ingredient is at least one of a diluent, a binder, a filler, a lubricant, a disintegrant, a penetration enhancer, a mucoadhesive, a taste enhancer, and a sweetening agent and mouthfeel enhancer.

Also provided is a use of a pharmaceutically effective dose of about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate and at least one non-medicinal ingredient, for increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine, wherein the stabilized nanoparticles of epinephrine bitartrate having a mean size (Z-average) ranging from about 279 nm to about 196 nm.

Also provided is a use of a pharmaceutically effective dose of about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate and at least one non-medicinal ingredient, for the manufacture of a composition for increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine, wherein the stabilized nanoparticles of epinephrine bitartrate having a mean size (Z-average) ranging from about 279 nm to about 196 nm.

Also provided is a pharmaceutical composition described herein, for use in

increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine.

Also provided is a use of a pharmaceutical composition described herein, for increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine.

Other objectives and advantages of this invention will become apparent from the following description taken in conjunction with the accompanying drawings, wherein are set forth, by way of illustration and example, certain embodiments of this invention. The drawings constitute a part of this specification and include exemplary embodiments of the present invention and illustrate various objects and features thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete understanding of the present invention may be obtained by references to the accompanying drawings when considered in conjunction with the subsequent detailed description. The embodiments illustrated in the drawings are intended only to exemplify the invention and should not be construed as limiting the invention to the illustrated embodiments.

FIG. 1 is a graph showing mean $\pm$ SD plasma epinephrine concentration versus time plots.

FIG. 2 is a HPLC Chromatogram of epinephrine bitartrate from the stored epinephrine tablets for 7 years.

FIG. 3 is a graph showing mean $\pm$ SD epinephrine tablet content (%) after storage at three storage conditions for three tablet formulations.

FIG. 4 is a graph showing mean $\pm$ SD epinephrine content (%) in 10 mg epinephrine tablets after storage at three storage conditions (25°, 5°C, and 5°C-N₂) for 6 months, 12 months, and 7 years.

FIG. 5 is a graph showing mean $\pm$ SD epinephrine content (%) in 20 mg epinephrine tablets after storage at three storage conditions (25°, 5°C, and 5°C-N₂) for 6 months and 12 months.

FIG. 6 is a graph showing mean $\pm$ SD epinephrine content (%) in 40 mg epinephrine tablets after storage at three storage conditions (25°, 5°C, and 5°C-N₂) for 18 months and 7 years.

FIG. 7 is a graph showing mean $\pm$ SD (n=3) absorptivity of oxidative products of epinephrine from 10 mg, 20 mg, and 40 mg epinephrine tablets stored at three storage conditions (25°, 5°C, and 5°C-N₂) for 7 years.

FIG. 8 is a graph showing mean $\pm$ SD (n=3) size of epinephrine bitartrate (Z-Average, nm) for several concentrations (0.7 mg/ml, 1.4 mg/ml, 2.8 mg/ml, 3.5 mg/ml, and 4.5 mg/ml) following several passes at 30 KPsi.

FIG. 9 is a graph showing mean (n=3) particle size distribution and zeta potential of 0.7 mg/ml epinephrine bitartrate following several passes at 30 KPsi.

FIG. 10 is a graph showing mean (n=3) particle size distribution of 1.4 mg/ml epinephrine bitartrate following several passes at 30 KPsi.

FIG. 11 is a graph showing mean (n=3) particle size distribution and zeta potential of 2.8 mg/ml epinephrine bitartrate following several passes at 30 KPsi.

FIG. 12 is a graph showing mean (n=3) particle size distribution and zeta potential of 3.5 mg/ml epinephrine bitartrate following several passes at 30 KPsi.

FIG. 13 is a graph showing mean (n=3) particle size distribution and zeta potential of 4.5 mg/ml epinephrine bitartrate following several passes at 30 KPsi.

FIG. 14 is a graph showing reproducibility (n=5) of particle size distribution and zeta potential of 2.8 mg/ml epinephrine bitartrate following one pass at 30 KPsi.

FIG. 15 is a FTIR spectra of epinephrine bitartrate before and after processing using 2.8 mg/ml sample concentration processed at 30 KPsi.

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DETAILED DESCRIPTION OF THE INVENTION

For the purpose of promoting an understanding of the principles of the invention, reference will now be made to embodiments illustrated herein and specific language will be used to describe the same. It will nevertheless be understood that no
10 limitation of the scope of the invention is thereby intended. Any alterations and further modification in the described compositions and methods and any further application of the principles of the invention as described herein, are contemplated as would normally occur to one skilled in the art to which the invention relates.

15 EXAMPLE 1: Epinephrine Sublingual Tablets-*In Vivo* Studies

Epinephrine tablets were developed and evaluated as described in the related application; U.S. Patent Provisional Application No. 61/550,359, filed on October 21, 2011. The tablet identified as "Formulation 7" was used for the *in vivo* studies described herein.

20 The rate and extent of epinephrine absorption from the sublingual tablets were studied in a validated rabbit model using a parallel-dose design. The administration of various epinephrine dosage forms, the protocol of the animal study, and the extraction analysis of epinephrine from collected blood samples were previously described. See Rawas-Qalaji et al. *J Allergy Clin Immunol* 117(2):398-403 2006.
25 Results demonstrated that mean ($\pm$ SD) area under the curve (AUC), maximum plasma

concentration ($C_{\max}$), and time at $C_{\max}$ ($T_{\max}$) after administration of 40 mg epinephrine sublingual tablets and epinephrine intramuscular injections were not significantly different ($P > .05$). However, the sublingual tablet formulation resulted with a plasma epinephrine concentrations similar to those achieved using a 0.3 mg
5 intramuscular epinephrine injection in an animal model. See Rawas-Qalaji et al. *J Allergy Clin Immunol* 117(2):398-403 2006. Further, the sublingual epinephrine tablets have a shelf-life of approximately seven years.

The sublingual epinephrine tablets (40 mg) were developed by substitution of non-medicinal ingredients (NMIs). By incorporation of mannitol, microcrystalline
10 cellulose (Ceolus™, Asahi Kasei Company) and a disintegrant (Ludiflash®, BASF The Chemical Company) into the tablets, total epinephrine release within sixty seconds was achieved (assessed *in vitro*). (Rachid et al. *AAPS PharmSci Tech* 12(2):544-552 2011). By addition of citric acid, the bitter taste of epinephrine was masked (assessed by electronic tongue). (Rachid et al. *AAPS PharmSci Tech*
15 11(2):550-557 2010).

The rate and extent of epinephrine absorption from the sublingual epinephrine tablets was studied in a validated rabbit model using a parallel-dose design. The positive control was 0.3 mg intramuscular injection (of epinephrine) from a commercially-available EpiPen® (epinephrine auto-injector 0.3/0.15 mg, Dey
20 Pharma, L.P. Nappa, CA). The negative control was a sublingual tablet containing only non-medicinal ingredients (NMIs) (contained no epinephrine).

Tablets were placed under the tongue for two minutes. Blood samples were collected at frequent intervals through an indwelling arterial catheter. Epinephrine concentrations were measured using high-performance liquid chromatography
25 (HPLC) with electrochemical detection. Plasma concentration versus time data was

analyzed using standard pharmacokinetic equations and WinNonlin® program (Pharsight Corporation).

- The mean $\pm$ SD maximum plasma concentration ($C_{\max}$) of 31.7 ± 10.1 ng/mL at a peak time ($T_{\max}$) of twenty minutes and an area under the curve (AUC) of 678.0 ± 149.0 ng/mL/min after administration of sublingual epinephrine (40 mg) did not differ significantly from the $C_{\max}$ of 27.6 ± 7.0 ng/mL at a $T_{\max}$ of thirty minutes with an AUC of 592.0 ± 122.3 ng/mL/min after an epinephrine (0.3 mg) intramuscular injection in the thigh ($p \leq 0.05$).

10 Table 1.1: Epinephrine Sublingual Tablets-Formulation 7

Tablet Contents	Ingredients	Type	Weight (mg)	Percentage %
1	Active Ingredient	*epinephrine bitartrate	72.77	36.39
2	Filler	**Ceolus™, MCC (PH-M-06)	11.17	5.59
3	Filler	**Ceolus™, MCC (PH-301)	66.80	33.40
4	Filler	***Ludiflash® (88% mannitol)	34.10	17.05
5	Flavor	†Citric Acid	2.50	1.25
6	Disintegrant	‡L-HPC (LH-11)	8.66	4.33
7	Lubricant	Mg Stearate	4.0	2.00
	Tablet Weight		200.00	100.00

*Each tablet contained 72.77 mg Epinephrine bitartrate which is equivalent to 40 mg Epinephrine base.

**Ratio of MCC (Ph-301) and MCC (PH-M-06) was kept at 6:1.

- 15 ***Ludiflash® consists of an average of 88% mannitol, Ludiflash® at 17.05% will contain 15% mannitol.

†Ratio of Epinephrine bitartrate and citric acid was kept at 29:1.

‡Ratio of total MCC and L-HPC was kept at 9:1.

MCC (PH-M-06) particle size is 7 μ m. MCC (PH-301) particle size is 50 μ m.

- 20 Ludiflash® particle size is 200 μ m.

Tables 1.2A-C: Epinephrine bioavailability after sublingual administration of different epinephrine doses and epinephrine intramuscular injection into the thigh.

A) Dose A (EpiPen® 0.3 mg I.M. Injection)

<i>Rabbit Id</i>	<i>AUC_{0-60min} (ng/mL/min)</i>	<i>C_{baseline} (ng/mL)</i>	<i>C_{max} (ng/mL)</i>	<i>T_{max}</i>
2	574.6	5.6	29.7	30.0
9	823.6	4.2	37.3	30.0
10	473.7	3.7	21.6	30.0
4	590.5	5.3	33.4	30.0
11	579.4	6.0	24.1	30.0
12	510.1	9.0	19.4	30.0
Mean	592.0	5.6	27.6	30.0
SD	122.3	1.9	7.0	0.0

5

B) Dose B (Epinephrine 40 mg Sublingual Tablet)

<i>Rabbit Id</i>	<i>AUC_{0-60min} (ng/mL/min)</i>	<i>C_{baseline} (ng/mL)</i>	<i>C_{max} (ng/mL)</i>	<i>T_{max}</i>
3	811.3	2.0	37.9	20.0
6	863.7	3.9	39.5	10.0
8	541.9	3.9	18.3	30.0
11	561.4	5.5	23.4	20.0
12	611.7	9.9	39.1	20.0
Mean	678.0	5.0	31.7	20.0
SD	149.0	3.0	10.1	7.1

C) Dose C (Placebo Sublingual Tablet)

<i>Rabbit Id</i>	<i>AUC_{0-60min} (ng/mL/min)</i>	<i>C_{baseline} (ng/mL)</i>	<i>C_{max} (ng/mL)</i>	<i>T_{max}</i>
4	259.0	0.3	8.9	40.0
5	332.0	0.2	11.7	40.0
7	226.5	0.2	9.0	20.0
3	241.2	0.5	6.6	60.0
6	136.2	2.2	3.6	30.0
8	126.1	3.0	5.0	10.0
Mean	220.1	1.1	7.5	33.3
SD	78.0	1.2	3.0	17.5

10

Table 1.3: Plasma epinephrine concentration versus time plots after administration of epinephrine or placebo sublingually and after epinephrine intramuscular injection.

Mean ($\pm$ SD) Epinephrine Plasma Concentration (ng/mL)

Time (min)	Dose A	Dose B	Dose C
0	5.6 $\pm$ 1.9	5.0 $\pm$ 3.0	1.1 $\pm$ 1.2
5	4.8 $\pm$ 1.9	9.1 $\pm$ 4.0	2.9 $\pm$ 1.0
10	6.4 $\pm$ 1.5	16.3 $\pm$ 13.5	3.0 $\pm$ 1.3
15	11.5 $\pm$ 5.8	11.8 $\pm$ 5.4	2.1 $\pm$ 1.4
20	5.3 $\pm$ 2.5	24.6 $\pm$ 14.1	5.2 $\pm$ 2.9
30	27.6 $\pm$ 7.0	9.5 $\pm$ 5.2	3.5 $\pm$ 2.1
40	7.5 $\pm$ 6.3	9.5 $\pm$ 5.1	5.3 $\pm$ 4.3
60	3.6 $\pm$ 0.8	5.8 $\pm$ 3.5	2.4 $\pm$ 2.2

5

The results are promising in that the epinephrine sublingual tablet formulation has similar absorption time ($T_{\max}$) and plasma epinephrine concentration ($C_{\max}$) to intramuscular injection. FIG. 1 shows a graph plotting plasma epinephrine concentration versus time after administration of epinephrine intramuscular injection (Dose A) and after administration of epinephrine (Dose B) or placebo (Dose C) sublingually. Mean ($\pm$ SD) AUC, $C_{\max}$, and $T_{\max}$ after administration of 40 mg epinephrine sublingual tablets and epinephrine intramuscular injections were not significantly different ($P>.05$).

15 EXAMPLE 2: Long-Term Stability of Epinephrine Tablets

Need for Sublingual Epinephrine Tablets

For the emergency treatment of anaphylaxis, prompt intramuscular injection of epinephrine in thigh muscle is the drug of choice and it is the only available dosage

form for the first-aid emergency treatment (Kemp et al. *Allergy* 63(8):1061-1070 2008; McLean-Tooke et al. *BMJ* 327(7427):1332-1335 2003; Simons et al. *Curr Opin Allergy Clin Immunol* 10(4):354-361 2010; Soar et al. *Resuscitation* 77(2):157-169 2008). Epinephrine auto-injectors such as EpiPen[®], EpiPen Jr[®] (Dey Pharma L.P. Nappa, CA), Twinject 0.3 mg[®], and Twinject 0.15[®] (Shionogi Pharma, Inc. Atlanta, GA) are prescribed for the first-aid emergency treatment of anaphylaxis. However, self-injectable epinephrine is underutilized when anaphylaxis occurs due to several drawbacks (Simons, *FE Ann Allergy Asthma Immunol* 102(5):403-409 2009; Simons, *FER Ann Allergy Asthma Immunol* 94(5):534-538 2005), including a short shelf-life of approximately a year.

Epinephrine is extensively metabolized after oral administration by the catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver (Hoffman et al. *Neurotransmission: The Automatic and Somatic Motor Nervous Systems* In: Hardman et al. editors Goodman & Gilman's The Pharmacological Basis of Therapeutics 9th edition New York: McGraw-Hill Companies Inc. pages 115-153 2001). Drugs that can be absorbed sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Bredenberg et al. *Eur J Pharm Sci* 20(3):327-334 2003; Glover et al. *Nicotine Tob Res* 4(4):441-450 2002; Guez, S *Chem Immunol Allergy* 82:62-76 2003; Rawas-Qalaji et al. *J Allergy Clin Immunol* 117(2):398-403 2006; Rawas-Qalaji et al. *Biopharm Drug Dispos* 27(9):427-435 2006; Saxena et al. *Eur J Obstet Gynecol Reprod Biol* 125(1):109-113 2006 (Epub 2005)).

Thus, the sublingual route is a promising alternative route for epinephrine administration. The high vascularity of the sublingual mucosa and the low molecular

weight of epinephrine facilitate rapid absorption directly into the venous circulation through the sublingual and frenular veins.

Considering the above, epinephrine tablets, especially those with a shelf-life of more than a year, are pharmaceutically desirable.

- 5 This study was undertaken to evaluate the long term stability of epinephrine in the fast-disintegrating tablets described herein during manufacturing processes and after pre-determined periods of storage.

Summary of Methods and Results of Stability Studies

- Epinephrine tablets, developed and evaluated as described in the related applications (U.S. Utility Patent Application No. 11/672,503, filed on February 7, 10 2007 and U.S. Utility Patent Application No. 11/530,360, filed on September 8, 2006) were tested and reported to be stable for at least twenty months. This same tablet formulation was analyzed after seven years of storage.

- The analysis was performed using standard USP chromatography methods 15 using high performance liquid chromatography with ultraviolet (UV) detector. Any potential oxidative products were extracted and analyzed using UV spectrophotometer. Results demonstrated that mean epinephrine dose in all the stored tablets did not differ significantly from controls and were within USP compedial limits for tablet content. Also, the absorptivity of oxidative products was below 0.1 20 for tablets stored at 25° C and below 0.01 for all other tablets. Results show that epinephrine in these sublingual tablets remains stable for at least seven years.

- Epinephrine tablets (10 mg, 20 mg, and 40 mg) were manufactured by direct compression and tested for quality control. Tablets were stored in opaque containers with desiccants at 25° C, 5° C, or 5° C under nitrogen. Six or three tablets were 25 randomly selected from each storage condition at six and twelve months for 10 mg

and 20 mg tablets, twenty months for 40 mg tablets, and 7 years for 10 mg, 20 mg, and 40 mg tablets. Tablets were inspected visually for changes in color and analyzed by HPLC-UV and UV spectrophotometer for measurement of epinephrine content and absorbance of oxidative products of epinephrine after storage, respectively.

- 5 Remaining epinephrine dose and the absorptivity of oxidative products were calculated and statistically analyzed versus controls ($p < 0.05$).

Pale yellow discoloration was observed only in 40 mg epinephrine tablets stored for twenty months and seven years at 25° C. Mean epinephrine dose in all the stored tablets did not differ significantly from controls and were within USP
10 compedial limits for tablet content. Absorptivity of oxidative products was below 0.1 for tablets stored at 25° C and below 0.01 for all other tablets (0.2 is the USP limit for impurities).

Materials

- 15 (-)-Epinephrine (+) bitartrate, (-)-3,4-dihydroxy- α -
[(methylamino)methyl]benzyl alcohol (+)-tartrate (1:1) salt, was purchased from Sigma-Aldrich (St. Louis, MO). Ceolus[®] PH-301 (microcrystalline cellulose) with a mean particle size of 50 μ m was supplied by Asahi Kasei Chemicals Corp (Tokyo, Japan) and low-substituted hydroxypropyl cellulose (LH11) with a mean particle size
20 of 50 μ m was supplied by Shin-Etsu Chemical Co (Tokyo, Japan). Magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ).

In aqueous solutions, epinephrine is unstable in the presence of oxygen, light, heat, and neutral or alkaline pH values. Epinephrine decomposes into the pharmacologically toxic colored molecules, adrenochrome and adrenolutin, which can
25 be eventually oxidized to melanin (*Br Med J* 5760(2):486 1971; Connors et al.

Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists 2nd edition New York: Wiley-Interscience Publication 1986). As a powder, epinephrine base and epinephrine salt should be stored in cool, dry, and light-resistant containers according to USP (USP/NF. Official Monograph: Epinephrine. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc. 2008) and Material Safety Data Sheet (MSDS) (Material Safety Data Sheet: (-)-Epinephrine (+)-bitartrate salt. Saint Louis, MO: Sigma-Aldrich; [06/19/2009]; available from the Sigma-Aldrich catalog).

Manufacturing and Quality Control of Tablets

10 Three fast-disintegrating tablet formulations containing epinephrine bitartrate equivalents to 10 mg, 20 mg, and 40 mg epinephrine were manufactured by direct compression. These tablets were formulated using microcrystalline cellulose, low-substituted hydroxypropyl cellulose, and magnesium stearate as described (Rawas-Qalaji et al. *Drug Dev Ind Pharm* 33(5):523-530 2007; Rawas-Qalaji et al. *AAPS*
15 *PharmSci Tech* 7(2):Article 41 2006). The tablet weight was 150 mg. All excipients were used as supplied and kept under low humidity condition before mixing. The mixing process was performed in a nitrogen-preflushed opaque glass container using three-dimensional manual mixer (Inversina, Bioengineering AG, Wald, Switzerland). The nitrogen gas was obtained from a local supplier and was used as supplied. The
20 powder mixture of the three tablet formulations was compressed right after mixing at a pre-selected compression force for each tablet formulation based on our previous results (Rawas-Qalaji et al. *AAPS PharmSci Tech* 7(2):Article 41 2006) that ensures rapid tablet disintegration and wetting while retaining sufficient hardness to withstand shipping and handling.

All tablet formulations were tested for tablet weight variation, drug content uniformity, and friability using the harmonized USP methods and criteria (USP/NF. Physical Tests: Tablet Friability (1216). 26/21 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2003; USP/NF. Physical Tests: Uniformity of Dosage Units (905). 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008). Drug content was analyzed using a high performance liquid chromatography (HPLC) system with ultra violet (UV) detection (Waters Corp., Milford, MA) as described below and tablet friability was measured using USP Friability instrument (Pharma Test Apparatebau GmbH, Hainburg, Germany). Tablet hardness, disintegration time, and wetting time were tested as described below by randomly selecting six tablets from each formulation. The mean $\pm$ standard deviation (SD) and percentage of coefficient of variation (RSD%) were calculated.

Hardness (H): The H or the crushing tolerance of tablets was measured using an Erweka[®] hardness tester (Heusenstamm, Germany).

Disintegration Time (DT): A relatively simple method with rigorous conditions was developed to evaluate the DT of rapidly disintegrating tablets. Each individual tablet was dropped into 10 ml glass test tube (1.5 cm diameter) containing 2 ml distilled water, and the time required for complete tablet disintegration was observed visually and recorded using a stopwatch. The visual inspection was enhanced by gently rotating the test tube at a 45° angle, without agitation, to distribute any tablet particles that might mask any remaining undisintegrated portion of the tablets (Rawas-Qalaji et al. *Drug Dev Ind Pharm* 33(5):523-530 2007; Rawas-Qalaji et al. *AAPS PharmSci Tech* 7(2):Article 41 2006).

Wetting Time (WT): Tablet WT was measured by a procedure modified from that reported by Bi *et al.* (*Chem Pharm Bull.* 44(11):2121-2127 1996). The tablet was placed at the center of 2 layers of absorbent paper fitted into a rectangular plastic dish (11 cm X 7.5 cm). After the paper was thoroughly wetted with distilled water, excess
5 water was completely drained out of the dish. The time required for the water to diffuse from the wetted absorbent paper throughout the entire tablet was then recorded by using a stopwatch (Rawas-Qalaji *et al.* *Drug Dev Ind Pharm* 33(5):523-530 2007; Rawas-Qalaji *et al.* *AAPS PharamSci Tech* 7(2):Article 41 2006).

Analysis of Tablets for Epinephrine Content Uniformity: Ten tablets out of 30
10 tablets were randomly selected from each formulation. Each tablet was allowed to disintegrate in 2.0 mL solvent containing 0.1 M perchloric acid and 0.1 mM sodium metabisulfite to maintain the stability of epinephrine, vortexed to dissolve all the epinephrine in the tablet, and then centrifuged. Aliquots of 50 μ L were withdrawn and diluted to 2.0 mL by the solvent. Drug content was analyzed using 2690 Alliance-
15 WatersTM HPLC system with UV detection (Waters Corp., Milford, MA) according to USP method for epinephrine injection analysis (USP/NF. Official Monograph: Epinephrine Injection. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008). Calibration curve was linear over the range of 6.125 to 600.0 μ g/mL with correlation of coefficients (R^2) of > 0.99 . The coefficient of variation
20 (RSD%) of the system reproducibility at concentrations of 6.125 and 600 μ g/mL (n=5 each) were 1.07% and 0.40%, respectively. The intra- and inter-assay CV% were 0.40 and 0.70% (n=2) and 6.9 and 3.5% (n=6), respectively.

Storage and Sampling of Tablets for Stability Testing

Each of the three tablet formulations, 10 mg, 20 mg, and 40 mg, was divided into three equal portions and immediately stored in tightly closed, opaque, 1 oz capacity, polytetrafluoroethylene (PTFE) containers (Berry Plastics Corporation, Evansville, IN) with desiccants. The tablets were subjected to a series of conditions at which tablets are commonly stored. Container 1 was stored at 25 °C (room temperature), container 2 was stored at 5 °C (refrigerator temperature), and container 3 was flushed with nitrogen before being tightly closed and stored at 5 °C. For the analysis of epinephrine content, six tablets were randomly sampled from the three containers after 6 and 12 months of storage for 10 and 20 mg tablets and after 20 months of storage for 40 mg tablets. After 7 years of storage, 3 tablets were sampled from the three containers of all tablets for the analysis of both epinephrine content and oxidative products. For the tablets stored under nitrogen at 5 °C (container 3), the containers were re-flushed with nitrogen after sampling, before being sealed and stored for the next sampling time.

Evaluation of Tablet Stability After Storage

The sampled tablets were visually examined for any color changes after storage and analyzed for epinephrine content by HPLC, and the UV absorbance of oxidative products were measured by spectrophotometer.

HPLC Analysis of Epinephrine Content: The sampled tablets from each formulation and storage condition were analyzed individually. Epinephrine and any oxidative products were extracted, diluted and analyzed by HPLC-UV using the same

procedure under “*Analysis of Tablet for Epinephrine Content Uniformity*” in a previous section.

UV Absorbance of Oxidative Products: Three tablets were allowed to disintegrate and were vortexed to dissolve all the epinephrine bitartrate and any
5 oxidative products in diluted hydrochloric acid (1 in 200) and then centrifuged. The supernatant was visually inspected against white background for any discoloration (USP/NF. Official Monograph: Epinephrine Injection. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008) and then filtered and diluted to make 4 mg/mL. The absorbance of diluted supernatant was measured at wavelength
10 310 nm using Genesys 10-s UV spectrophotometer (Thermo Fisher Scientific, Madison, USA) according to the USP method and criteria for the limit of adrenalone in epinephrine bitartrate solution (USP/NF. Official Monograph: Epinephrine Bitartrate. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008). The absorbance of epinephrine bitartrate standard in the same solution and at
15 the same concentration was used as a first control (control 1). The second control (control 2) was prepared to take in consideration the absorbance of any water-soluble excipients from the tablet that may interfere with the UV absorbance of epinephrine bitartrate oxidative products. Placebo tablets were dissolved and diluting using the same solvent and procedure above, then spiked with epinephrine bitartrate standard to
20 make 4 mg/mL concentration. The absorptivity of all samples and controls was calculated by dividing the absorbance by the product of the concentration of the drug (g/L) and the path length (cm) of the cell used to measure the absorbance (USP/NF. Physical Tests: Spectrophotometry and Light-Scattering (851). 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008). An absorptivity of not
25 more than 0.2 was considered acceptable according to the USP criteria for the limit of

adrenalone in epinephrine bitartrate solution (USP/NF. Official Monograph: Epinephrine Bitartrate. 31/26 ed. Rockville, MD: United States Pharmacopoeial Convention, Inc.; 2008).

Data Analysis

5 The mean epinephrine content (dose) at T₀ for the manufactured tablets formulations before storage was obtained from the content uniformity test and was used as a control. The epinephrine contents (doses) in the sampled tablets for the three formulations and from each storage condition at various time-points and in control tablets were statistically compared by two-way ANOVA and Tukey-Kramer tests
10 using statistical analysis software NCSS (NCSS, Kaysville, UT). Differences were considered significant at $p < 0.05$. The epinephrine content (%) in the sampled tablets was calculated using the mean of epinephrine content in control tablets.

 The absorptivity of oxidative products of epinephrine in the tablets from each formulation at each storage condition and in controls was statistically compared by
15 two-way ANOVA and Tukey-Kramer tests.

 The significance of existing trends for the stability of epinephrine or the increase of its oxidative products associated with the dose in the stored tablets was also tested.

Results

 Hardness, disintegration time, and wetting time for the three tablet formulations,
20 10 mg, 20 mg, and 40 mg, are shown in Table 2.1.

Table 2.1- The hardness, disintegration time, and wetting time of the 10 mg, 20 mg, and 40mg tablet formulations *†

In vitro Tablet Characteristics

Formulations	H	CV	DT	CV	WT	CV
10 mg tablets	3.1 ± 0.1	6.4	7.7 ± 0.3	9.7	24.2 ± 0.9	8.8
20 mg tablets	2.9 ± 0.1	11.6	12.0 ± 0.6	11.8	41.8 ± 3.6	21.1
40 mg tablets	2.4 ± 0.1	12.4	13.5 ± 0.2	4.1	26.17 ± 1.8	17.0

* mean ± SD (n=6)

† H indicates tablet hardness (kg); CV, coefficient of variation (%); DT, disintegration
5 time (sec); WT, wetting time (sec).

All of the three tablet formulations were within USP specifications for weight
variation, drug content uniformity, and friability (USP/NF. Physical Tests: Tablet
Friability (1216). 26/21 ed. Rockville, MD: United States Pharmacopeial Convention,
10 Inc.; 2003; USP/NF. Physical Tests: Uniformity of Dosage Units (905). 31/26 ed.
Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008). The mean ± SD
epinephrine doses in control tablets (at T₀) obtained from content uniformity test for
10 mg, 20 mg, and 40 mg epinephrine tablet formulations were 9.8±0.4 mg, 20.1± 1.5
mg, and 38.0±1.8 mg, respectively.

15 There were no detectable visual changes in the 10 mg and 20 mg epinephrine
tablet formulations stored for 6 and 12 months under the three storage conditions.
Also, there were no detectable visual changes in the 40 mg epinephrine tablet
formulation stored for 18 months at 5 °C with and without nitrogen flushing prior to
storage. Pale yellow tablet discoloration was observed only in the 40 mg epinephrine
20 tablet formulation stored for 18 months at 25 °C. However, no yellow discoloration
was visually detected against white background for the supernatant solution from the

dissolved epinephrine tablets for UV absorbance measurements and for HPLC analysis.

Mean ($\pm$ SD) epinephrine contents (dose and %) in the 10 mg and 20 mg epinephrine tablet formulations stored for 6 months, 12 months, and 7 years (only 10 mg tablet), and in the 40 mg epinephrine tablet formulation stored for 18 months and 7 years at 25 °C, 5 °C, and 5 °C with nitrogen flushing are shown in Tables 2.2A-B and 2.3, respectively.

Tables 2.2A-B below show mean $\pm$ SD epinephrine content remaining in 10 mg (Table 2.2A) and 20 mg (Table 2.2B) epinephrine tablets stored at 25°C, 5°C, and 5°C with nitrogen flushing (5°C-N₂) for 6 months, 12 months, and 7 years (only 10 mg tablets).

Tables 2.2A-B: Epinephrine Content

Table 2.2A: 10 mg epinephrine tablets; Mean $\pm$ SD content, mg (%)[†]

Time	25°C	5°C	5°C-N ₂
0	9.8 $\pm$ 0.4 (100)		
6 Months*	9.2 $\pm$ 0.3 (95 $\pm$ 3)	9.3 $\pm$ 0.4 (95 $\pm$ 4)	9.4 $\pm$ 0.7 (96 $\pm$ 7)
12 months*	9.6 $\pm$ 0.3 (98 $\pm$ 3)	9.7 $\pm$ 0.6 (99 $\pm$ 6)	9.6 $\pm$ 0.3 (98 $\pm$ 3)
7 years**	8.9 $\pm$ 0.1 (91 $\pm$ 1)	9.6 $\pm$ 0.1 (98 $\pm$ 1)	8.9 $\pm$ 0.4 (91 $\pm$ 4)

Table 2.2B: 20 mg epinephrine tablets; Mean $\pm$ SD content, mg (%)[†]

Time	25°C	5°C	5°C-N ₂
0	20.1 $\pm$ 1.5 (100)		
6 Months*	19.8 $\pm$ 1.1 (98 $\pm$ 6)	19.8 $\pm$ 1.2 (98 $\pm$ 6)	20.3 $\pm$ 0.8 (101 $\pm$ 4)
12 months*	19.4 $\pm$ 0.9 (96 $\pm$ 5)	20.3 $\pm$ 0.7 (101 $\pm$ 3)	20.9 $\pm$ 1.9 (104 $\pm$ 9)
7 years**	NA	NA	NA

*n=6

**n=3

†Epinephrine content (%) was calculated from mean epinephrine content in control tablets at T₀. Epinephrine content in all tablets was not significantly different from controls at the different storage conditions, $p > 0.05$, and was within USP compendial limits for tablet content (85-115%).

5

Table 2.3 below shows mean $\pm$ SD epinephrine content remaining in 40 mg epinephrine tablets stored at 25°C, 5°C, and 5°C with nitrogen flushing (5°C-N₂) for 18 months and 7 years.

10

Table 2.3: Epinephrine Content

Table 2.3: 40 mg epinephrine tablets; Mean $\pm$ SD content, mg (%)†

Time	25°C	5°C	5°C-N ₂
0	38.0 $\pm$ 1.8 (100)		
18 months*	37.5 $\pm$ 0.5 (99 $\pm$ 1)	38.9 $\pm$ 1.5 (103 $\pm$ 4)	38.5 $\pm$ 2.9 (101 $\pm$ 8)
7 years**	37.7 $\pm$ 1.0 (99 $\pm$ 3)	38.1 $\pm$ 0.8 (100 $\pm$ 2)	36.9 $\pm$ 0.3 (97 $\pm$ 1)

*n=6

**n=3

15 †Epinephrine content (%) was calculated from mean epinephrine content in control tablets at T₀. Epinephrine content in all tablets was not significantly different from controls at the different storage conditions, $p > 0.05$, and was within USP compendial limits for tablet content (85-115%).

20 Epinephrine dose in the three tablet formulations at the three storage conditions did not differ significantly from control tablets over 7 years for 10 mg and 40 mg tablet formulations and 12 months for 20 mg tablet formulation. Also, the epinephrine content (%) for all the tested tablets was within the harmonized USP compendial limits, acceptance value (AV) < L1 (15%).

There was no change in the shape of epinephrine peaks or a presence of a second peak in the HPLC chromatogram that indicates for the degradation of epinephrine or the existence of oxidative products in the analyzed tablets after storage (FIG. 2).

5 There was no significant trend in the stability of epinephrine associated with increasing epinephrine dose in the stored tablets at the various storage conditions (FIG. 3). FIG. 3 is a graph showing mean $\pm$ SD (n=6) epinephrine tablet content (%) after storage at three storage conditions for three tablet formulations. Only tablets stored for 12 months and 18 months were plotted). $p > 0.05$.

10 Tablet content (%) of 10 mg epinephrine tablets after storage at three storage conditions (25°, 5°C, and 5°C-N₂) for 6 months, 12 months, and 7 years is shown in FIG. 4.

Tablet content (%) of 20 mg epinephrine tablets after storage at three storage conditions (25°, 5°C, and 5°C-N₂) for 6 months and 12 months is shown in FIG. 5.

15 Tablet content (%) of 40 mg epinephrine tablets after storage at three storage conditions (25°, 5°C, and 5°C-N₂) for 18 months and 7 years is shown in FIG. 6.

In FIGS. 4-6, data was plotted using mean $\pm$ SD and epinephrine content was not significantly different from control tablets, $p > 0.05$, and was within USP compedial limits for tablet content (85-115%).

20 The absorptivity (mean $\pm$ SD) of epinephrine tablets from each formulation stored at each storage condition for 7 years are shown in Table 2.4.

Table 2.4: Absorptivity of Oxidative Products of Epinephrine (Mean $\pm$ SD)*

Formulations	25°C	5°C	5°C-N ₂
Epinephrine Bitartrate Solution (control 1)	0.001 $\pm$ 0.000		
Epinephrine Tablets at T ₀ (control 2)	0.001 $\pm$ 0.000		
10 mg tablets	0.063 $\pm$ 0.001 ^a	0.003 $\pm$ 0.000 ^b	0.002 $\pm$ 0.000
20 mg tablets	0.069 $\pm$ 0.001 ^a	0.003 $\pm$ 0.000 ^b	0.002 $\pm$ 0.000
40 mg tablets	0.032 $\pm$ 0.001 ^{a,c}	0.001 $\pm$ 0.001	0.002 $\pm$ 0.0001

*n=3

^ap < 0.05, from controls (1 and 2) and epinephrine tablets stored at 5°C and 5°C-N₂.

5 ^bp < 0.05, from controls (1 and 2).

^cp < 0.05, from 10 mg and 20 mg tablets stored at 25°C.

The absorptivity of oxidative products of epinephrine in the tablets from each formulation stored at each storage condition was below 0.2. However, the absorptivity of epinephrine tablets stored at 25 °C were significantly higher than controls and
10 tablets stored at 5 °C and 5 °C-N₂.

However, the absorptivity of 10 mg and 20 mg epinephrine tablets, but not the 40 mg tablets, stored at 5 °C were significantly higher than controls. Also, the absorptivity of 10 mg and 20 mg epinephrine tablets stored at 25 °C were significantly higher than 40 mg epinephrine tablets (FIG. 7). FIG. 7 is a graph showing
15 absorptivity of three epinephrine tablet formulations after storage at three storage conditions for 7 years. Data was plotted using mean $\pm$ SD (n=3). *p < 0.05, from controls 1 and 2, **p < 0.05, from epinephrine tablets stored at 5° C and 5° C-N₂, ***p < 0.05, from 10 mg and 20 mg epinephrine tablets stored at 25° C. However, all tablets were below 0.2 (USP limit for impurities).

20

Discussion of Experiment and Results

Epinephrine in a dosage form exists only as ampoules for intravenous, intramuscular, or subcutaneous administration and prefilled autoinjectors for intramuscular self-administration by the patient for the emergency treatment of anaphylaxis. There is no tablet dosage form for epinephrine that exists in the market. Thus, there are no specific pharmacopeial guidelines or testing criteria for epinephrine tablet dosage form. The general harmonized USP compendial limits for tablet content is 85-115% of the label claim (the acceptance value (AV) $\leq$ the maximum allowed acceptance value (L1), which is 15%) (USP/NF. Physical Tests: Uniformity of Dosage Units (905). 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008).

The stability of epinephrine in solutions has been thoroughly investigated, and the optimal pH, storage conditions, and the required quantities of antioxidants to stabilize epinephrine have been determined (Connors et al., 1986, in Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists, Wiley-Interscience Publication: New York; Rawas-Qalaji et al. *Ann Allergy Asthma Immunol* 102(6):500-503 2009). The recommendations for the storage of epinephrine as a powder are documented in the USP (USP/NF. Official Monograph: Epinephrine Injection. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008) and stated in the Material Safety Data Sheet (MSDS) (Material Safety Data Sheet: (-)-Epinephrine (+)-bitartrate salt. Saint Louis, MO: Sigma-Aldrich; [06/19/2009]). Although it would be expected that epinephrine should be stable for a longer period of time as a solid state more than a solution, there is no data about the time-frame for the stability of epinephrine powder at these recommended storage conditions. Also, there is no data about the stability of epinephrine in a tablet dosage form and whether the

manufacturing processes and excipients selected for the formulation of these epinephrine tablets affect the stability of epinephrine on the long term.

The storage conditions that were selected for these experiments were based on the most common places for tablets to be stored at. However, dark and moisture-
5 controlled packaging stored at room temperature is the most convenient packing material and place to store epinephrine tablets since these tablets are expected to be carried by allergic patients at all times. Epinephrine is sensitive to light, moisture, temperature, and oxygen (Gelone et al. Pharmaceutical and Medicinal Agents. In: Gerbino, P. editor. Remington: The Science and Practice of Pharmacy 21st edition,
10 Baltimore, MD: Lippincott Williams & Watkins; page 1386, 2005). Opaque containers containing desiccants were selected for storing the tablets during the experiments to control for the effect of light and moisture on epinephrine stability. The long-term effect of temperature and oxygen on the stability of epinephrine tablets were evaluated in these experiments.

15 It was reported that the hydroperoxide content in some of the commonly used excipients in pharmaceutical formulations may contribute to oxidative reactions in labile medications. Temperature increase was also found to increase the hydroperoxide content in these excipients. Microcrystalline cellulose, which is the main excipient used for the formulation of epinephrine tablets, however, was reported
20 to contain minute levels of hydroperoxide (< 10 nmole/g) and these levels did not increase after being exposed to elevated temperatures for four weeks (Wasylaschuk et al. *J Pharm Sci* 96(1):106-116 2007). Thus, the low content of hydroperoxide in the epinephrine tablet formulation might play an additional role to reduce epinephrine oxidation.

The results from the content uniformity test suggest that the manufacturing procedures and processes used in these experiments did not affect the stability of epinephrine in this tablet formulation. Direct compression method is commonly used for manufacturing tablets containing light, heat, or moisture-sensitive active pharmaceutical ingredient (Carlin, BAC. Direct Compression and the Role of Filler-Binders. In: Augsburger LL, Hoag SW, editors. Pharmaceutical Dosage Forms: Tablets. New York, NY: Informa Healthcare USA, Inc. pages 173-216 2008). Also considering other measures like selecting excipients with low moisture content, maintaining the excipients under low humidity before mixing, using opaque containers flushed with nitrogen before mixing and storage, and compressing the powder mixture right after mixing might play a role as well in minimizing any oxidative reactions before and during the manufacturing of the tablets and during their storage.

The initial results from the HPLC analysis for the epinephrine content in 10 mg and 20 mg tablets showed that epinephrine was stable in this tablet formulation for up to 12 months (FIGS. 4 and 5) at the three storage conditions, 25 °C (room temperature), 5 °C (refrigerator temperature), and 5 °C-N₂ (refrigerator temperature and under nitrogen). Also, there was no tablet discoloration observed after 12 months of storage. As a result, the storage time for the 40 mg epinephrine tablets was extended for additional 6 months because it was anticipated that they could be more susceptible for degradation over a longer storage time more than the previous ones, *i.e.* 10 mg and 20 mg tablets, due to higher epinephrine content in these tablets.

The 40 mg epinephrine tablets were also stable after 18 months of storage at the three storage conditions (FIG. 6). Thus, the storage time was extended again for a total of 7 years for the lowest and highest doses, 10 mg and 40 mg epinephrine tablets.

The HPLC analysis of these tablets at the three storage conditions showed that epinephrine was stable in this tablet formulation (FIGS. 4 and 6).

However, the 40 mg epinephrine tablets stored at 25 °C resulted in a pale or slightly yellow discoloration of the tablets that can be an indication for initial degradation of epinephrine and the formation of adrenolutin, a bright yellow oxidative product of epinephrine. (Bacq, *ZM Pharmacol Rev* 1:1-26 1949; Dhalla et al. *Mol Cell Biochem* 87(1):85-92 1989). From the statistical perspective, the epinephrine dose remaining in these tablets did not differ significantly from control tablets and was with the USP compendial limits for content uniformity.

10 A discoloration of epinephrine solution into pinkish color, due to oxidation to adrenochrome, or darker than slightly yellow color, due to oxidation to adrenolutin, or the presence of precipitate, due to conversion of oxidative products to melanin (*Br Med J* 5760(2):486 1971; Connors et al. Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists 2nd edition New York: Wiley-Interscience Publication
15 1986; Bacq, *ZM Pharmacol Rev* 1:1-26 1949), is not acceptable according to the USP standards for epinephrine injections (USP/NF. Official Monograph: Epinephrine Injection. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008). However, the detection of only yellow discoloration upon visual inspection of epinephrine solution from epinephrine injection dosage form requires the
20 measurement of its UV absorbance to make sure that it does not exceed the absorbance of its standard solution. Since epinephrine tablets do not exist in the market, there is no specific pharmacopeial procedure in place for testing the color and clarity of epinephrine bitartrate from epinephrine tablets or for testing the limits of adrenolutin in epinephrine tablet dosage form. Therefore, the measurement of UV
25 absorbance of epinephrine bitartrate and its oxidative products from a tablet dosage

form was performed according to the USP procedure and limit for adrenalone in epinephrine bitartrate as described earlier under Evaluation of Tablet Stability After Storage section. Adrenalone is an intermediate product for the epinephrine synthesis (Stolz, AF Chem Ber 37:4152 1904).

5 Despite of the lack of yellow discoloration of the supernatant solution for the 40 mg epinephrine tablets stored at 25 °C and although the tablets' discoloration was not darker than slight yellow color, their absorptivity and the absorbitivity of all the stored tablets were measured to further investigate the stability of epinephrine in the tablet formulation and to identify the existence of any trend in the stability at the various
10 storage conditions and doses.

 The absorptivity results of all the stored epinephrine tablets were below the USP limits for adrenalone in epinephrine bitartrate solution, which is 0.2, even for the tablets stored at 25 °C. Therefore, the lack of discoloration and the low absorptivity of epinephrine bitartrate solution from epinephrine tablets, and the absence of a second
15 peak for adrenolutin in the HPLC spectrum (Dhalla et al. *Mol Cell Biochem* 87(1):85-92 1989) supported by the high epinephrine content in the tablets suggest that the oxidative products of epinephrine in these tablets were insignificant or absent.

 Although the epinephrine content (%) data from the different epinephrine tablets (10 mg, 20 mg, and 40 mg) stored at the same condition were not significantly
20 different from each other (FIG. 3) and may not reflect any effect for the epinephrine dose used in the tablets on its own stability, the abosrpitivity data were more discriminative despite that the absorptivity of all the stored tablets at the different storage conditions were far below 0.2 (FIG. 7).

For the tablets stored at 25 °C, although the absorptivity of all epinephrine tablets were significantly higher than controls due to the effect of temperature and oxygen on epinephrine stability, the absorptivity of 40 mg epinephrine tablets was significantly lower than 10 mg and 20 mg tablets (FIG. 7). For the tablets stored at 5 °C, the absorptivity of only 10 mg and 20 mg epinephrine tablets were significantly higher than controls, which represents only the effect of exposing epinephrine tablets to oxygen (FIG. 7). For the tablets stored at 5 °C-N₂, there was no significant difference in the absorptivity between 40 mg epinephrine tablets and 10 mg and 20 mg tablets. Also the absorptivity of all these tablets was not different from controls, which clearly was due to the protection of epinephrine tablets from temperature and oxygen (FIG. 7).

Also, it indicates that the use of opaque containers and desiccants in the containers were sufficient to reduce the exposure of epinephrine tablets to light and humidity, respectively, which helped in maintaining the stability of epinephrine as well.

Despite the lack of significant trend for the effect of epinephrine dose on the epinephrine absorptivity and thus the stability of epinephrine, these observations may suggest that higher epinephrine content in the tablets stored at less optimal storage conditions reduces the susceptibility of its own degradation. Also, the results from this study shows that epinephrine in this fast-disintegrating tablet formulation are stable for at least 7 years at less optimal storage conditions. However, limiting the exposure of these tablets to oxygen and more importantly storing them at lower temperatures will reduce epinephrine oxidation. Further studies are useful to evaluate the stability of these epinephrine tablets at 25 °C under N₂.

25

EXAMPLE 3: Fabrication and Characterization of Epinephrine Nanoparticles Using High Shear Fluid Processor (Microfluidizer)

The feasibility of the fabrication of epinephrine nanoparticles using a high shear fluid processor (microfluidizer) was described in prior patent applications; U.S.

5 Provisional Patent Application No. 61/550,359, filed on October 21, 2011 and International Application No. PCT/US2011/26604, filed on March 1, 2011.

Epinephrine IM (intramuscular) injection in the thigh is the recommended route of administration for first aid treatment of anaphylaxis in the community. Due to drawbacks of this injection, alternative methods of administration are being
10 explored.

The instant inventors developed a fast-disintegrating epinephrine tablet suitable for SL (sublingual) administration (Rawas-Qalaji et al. *AAPS Pharm Sci Tech* 7:E41 2006). Sublingual administration of 40 mg epinephrine as the bitartrate salt using these tablets resulted in a rate and an extent of epinephrine absorption similar to
15 that achieved following intramuscular injections of 0.3 mg epinephrine in the thigh (Rawas-Qalaji et al. *J Allergy Clin Immunol* 117:398-403 2006). Sublingual doses ranging from 5 to 40 mg epinephrine as the bitartrate salt were studied to achieve equivalent plasma concentrations.

Without being bound by theory, it is thought that by fabricating epinephrine
20 into nanoparticles and incorporating penetration enhancers and mucoadhesives (if needed) into the tablet formulation, absorption of sublingually-administered epinephrine will significantly increase and will result in reduction of the sublingual epinephrine dose required.

Fabrication of Nanoparticles

Nanoparticles were fabricated from epinephrine base and epinephrine bitartrate (Bit) using high energy fluidization (microfluidization) techniques. These techniques involve the use of various concentrations in various solvents, particularly water and isopropanol, at various temperatures and pressures ranging from about 5 8,000 psi to 30,000psi and to about 8.3° to 43.3°C under various passes. Particle size was measured before size reduction using a Mastersizer (Malvern) and/or a NiComp 370 Submicron Particle Sizer (NiComp) and nano-sized particles were confirmed using laser diffraction techniques (Zetasizer, Malvern). The particles were lyophilized (freeze-dried) using a bench top lyophilizer (ART Inc.) or dried by speed 10 vacuum concentrator.

Methods

Selection of Vehicle for Shear Fluid Processing: The carrier vehicle for epinephrine base or epinephrine bitartrate was selected based on least solubilizing capacity at room temperature and toxicity of that vehicle. Two milligrams of 15 epinephrine bitartrate was dissolved in 1 ml methanol, isopropyl alcohol, acetonitrile, acetone, hexane, chloroform, or tetrahydrofuran, ethyl acetate by vortexing for 15 minutes. Supernatant solution was withdrawn and filtered through 0.22 µm nylon syringe filters to be analyzed for the quantification of dissolved epinephrine.

High Performance Liquid Chromatography (HPLC) Analysis: Samples from 20 the solubility study were analyzed using a PerkinElmer HPLC system with ultraviolet (UV) detector and Econspher C₁₈, 4.6 x 150 mm, 3 µm column (Alltech). Analysis and condition were performed according to USP 26th edition, 2003 “Epinephrine Injection Monograph.”

Nanoparticles Fabrication: Epinephrine bitartrate or base was suspended in isopropyl alcohol at various concentrations to make 6 mL samples (0.7, 1.4, 2.8, 3.5, and 4.5 mg/ml). Samples were prepared in triplicates for each concentration, n=3. All prepared samples were processed using LV-1 High Shear Fluid Processor

5 “Microfluidizer” (Microfluidics, Newton, MA) equipped with G10Z reaction chamber under various pressures ranging from 15 to 30 KPsi for single or several passes. The microfluidizer-receiving coil was immersed in ice to reduce the heat produced during the process for heat-sensitive drugs. The particle size and potential of the produced nanosuspension were measured. The nanosuspension was centrifuged at 15,000 rpm

10 and 15°C for 30 minutes to collect the nanoparticles, and the solvent was then removed. The collected nanoparticles were completely dried by speed vacuum concentrator or lyophilization.

Nanoparticle Characterization: The particle size distribution and zeta potential of the nanosuspension was measured before and after size reduction by

15 volume and by intensity using Mastersizer (Malvern) and Zetasizer Nano-ZS90 (Malvern), respectively. The average of 3 measurements for each sample was reported. Stability of epinephrine and epinephrine bitartrate was monitored by visually inspecting the color of the processed suspension and by using Fourier Transform InfraRed (FT-IR, PerkinElmer Inc) for the dried samples.

20

Results

Selection of Vehicle for Shear Fluid Processing: In order to determine suitable vehicles to suspend epinephrine base or epinephrine bitartrate (Bit) for nanoparticle

fabrication, solubility studies were carried out to select the vehicles that minimally solubilize the drug.

5

Table 3.1 Solubility

Sample Name	Amount Dissolved ($\mu\text{g/ml}$)
Epinephrine Base solubility in water	53.37
Epinephrine Bit solubility in methanol	209.01
Epinephrine Bit solubility in isopropyl alcohol	12.30
Epinephrine Bit solubility in acetonitrile	15.46
Epinephrine Bit solubility in acetone	31.28
Epinephrine Bit solubility in hexane	0.53
Epinephrine Bit solubility in chloroform	1.87
Epinephrine Bit solubility in tetrahydrofuran (THF)	155.18
Epinephrine Bit solubility in ethyl acetate	12.60

Results of the Fabrication of Epinephrine Bitartrate Nanoparticles a): Mean size of epinephrine bitartrate (Z-Average) following several passes at 30 KPsi is shown below in Table 3.2 and in FIG. 8. FIG. 8 is a graph showing Mean $\pm$ SD (n=3) size of epinephrine bitartrate (Z-Average, nm) for several concentrations (0.7 mg/ml, 1.4 mg/ml, 2.8 mg/ml, 3.5 mg/ml, and 4.5 mg/ml) following several passes at 30 KPsi.

15

Table 3.2: Mean $\pm$ SD* particles size distribution of epinephrine bitartrate (Z-Average,
 5 nm, and pdi) for several concentrations (0.7 mg/ml, 1.4 mg/ml, 2.8 mg/ml, 3.5 mg/ml,
 and 4.5 mg/ml) following several passes at 30 KPsi.

Mean $\pm$ SD* particles size (nm) and polydispersity (pdi)

Number of Passes	0.7 mg/ml	1.4 mg/ml	2.8 mg/ml	3.5 mg/ml	4.5 mg/ml
0†	131,800 $\pm$ 10500	131,800 $\pm$ 10500	131,800 $\pm$ 10500	131,800 $\pm$ 10500	131,800 $\pm$ 10500
1	706 $\pm$ 289(0.257 $\pm$ 0.060)	665 $\pm$ 47(0.239 $\pm$ 0.044)	249 $\pm$ 38(0.490 $\pm$ 0.134)	1211 $\pm$ 389(0.335 $\pm$ 0.112)	1091 $\pm$ 43(0.321 $\pm$ 0.060)
2	879 $\pm$ 376(0.119 $\pm$ 0.052)	339 $\pm$ 34(0.158 $\pm$ 0.021)	827 $\pm$ 114(0.649 $\pm$ 0.236)	1418 $\pm$ 79(0.576 $\pm$ 0.080)	1242 $\pm$ 28(0.315 $\pm$ 0.055)
3	573 $\pm$ 329(0.167 $\pm$ 0.026)	422 $\pm$ 40(0.190 $\pm$ 0.022)	971 $\pm$ 124(0.243 $\pm$ 0.112)	1116 $\pm$ 135(0.506 $\pm$ 0.067)	
4	618 $\pm$ 267(0.217 $\pm$ 0.035)	456 $\pm$ 40(0.178 $\pm$ 0.039)	976 $\pm$ 163(0.219 $\pm$ 0.035)		
5	539 $\pm$ 65(0.170 $\pm$ 0.070)		1713 $\pm$ 36(0.099 $\pm$ 0.043)		
6	501 $\pm$ 34(0.195 $\pm$ 0.067)		1161 $\pm$ 127(0.836 $\pm$ 0.268)		

10 *n=3

† n=6, before processing, the 10th percentile (Dv0.1), median (Dv0.5), and 90th percentile (Dv0.9) were 39.8 $\pm$ 3.0 μ m, 113.6 $\pm$ 9.1 μ m, and 254.8 $\pm$ 20.1, respectively.

Epinephrine bitartrate size reduction resulted in various sizes based on the
 15 concentration of the suspension processed. Samples at lower concentrations required
 several passes to reduce the particle size; however, samples at higher concentrations
 were very condensed to be processed and required several passes before particle size
 was reduced. Over processing the sample beyond the number of optimal passes
 required can result in aggregation of the reduced particles and an increase in their
 20 size. The optimal concentration for epinephrine bitartrate suspended in isopropyl
 alcohol (IPA) was 2.8 mg/ml that resulted in the lowest particle size and uniform
 narrow size range after only one pass.

Results of the Fabrication of Epinephrine Bitartrate Nanoparticles b): Particle size distribution and zeta potential of epinephrine bitartrate following several passes at 30 KPsi is shown in FIGS. 9-13.

At 0.7 mg/ml epinephrine bitartrate (n=3) in isopropyl alcohol (IPA), particles
5 were distributed over a wide size-range and resulted in less negative zeta potential due to high polydispersity despite several passes (FIG. 9).

At 1.4 mg/ml epinephrine bitartrate (n=3) in IPA, particle distribution was uniform and required two passes (at 30 KPsi) to achieve optimal size (FIG. 10).

At 2.8 mg/ml epinephrine bitartrate (n=3) in IPA, particle distribution was
10 uniform and particle size was optimal following one pass only, which is reflected in more negative zeta potential (FIG. 11). Further processing caused the particles to aggregate and increase and the distribution became less uniform.

At 3.5 mg/ml epinephrine bitartrate (n=3) in IPA, particle distribution was slightly not uniform the first two passes and particle size was at a higher range (FIG.
15 12). Further processing caused the particles to aggregate and increase and the distribution became even less uniform.

At 4.5 mg/ml epinephrine bitartrate (n=3) in IPA, particle distribution was considerably not uniform and particle size was in the higher range (FIG. 13).

Reproducibility of the Fabrication of Epinephrine Bitartrate Nanoparticles:

20 Five samples of 2.8 mg/ml epinephrine bitartrate in IPS were processed at 30 KPsi for one pass. Mean $\pm$ SD size of epinephrine bitartrate (Z-Average) was 230 $\pm$ 39 nm with a coefficient of variation (RSD) of 17% (Table 3.3). Mean $\pm$ SD particle size distribution was uniform and size was mainly (90 $\pm$ 8% with RSD of 9%) at the lower range (187 $\pm$ 34 nm with RSD of 18%) and mean $\pm$ SD zeta potential of epinephrine bitartrate
25 was -24 $\pm$ 7 mv (FIG. 14).

Table 3.3: Mean Size* of epinephrine bitartrate (Z-Average, nm) for 2.8 mg/ml concentration following one pass at 30 KPsi

Sample Number	Mean Size (Z-Average, nm)
1	263
2	279
3	196
4	199
5	211

*n=3 measurements

Mean	230
SD	39
RSD (%)	17

5

Stability of the Fabricates Epinephrine Bitartrate Nanoparticles: There was no change in color of the fabricated nanosuspension at the various concentrations. The FTIR (Fourier transform infrared) spectra of epinephrine bitartrate dried particles were similar before and after processing using 2.8 mg/ml sample concentration (FIG.

10 15).

Based upon the above results, it is evident that the fabrication of nanoparticles using a high shear fluid processor (Microfluidizer) was successful. The selection of optimal sample concentration to be processed at the optimal pressure resulted in reproducible, uniform, stable, and small particle size range. For epinephrine bitartrate, particles were reduced from $150.7 \pm 5 \mu\text{m}$ to $223 \pm 35 \text{ nm}$ at sample concentration of 2.8 mg/ml and pressure of 30 KPsi that was produced for only one pass.

20

Conclusions Based on Results of Experiments of EXAMPLES 1-3

Fast-disintegrating sublingual epinephrine tablets (disclosed in related application: U.S. Utility Patent Application No. 11/530,360, filed on September 8, 2006) which retain sufficient hardness to withstand shipping and handling and
5 disintegrate to release epinephrine rapidly (< 15 sec) (Rachid et al. *AAPS PharmSci Tech* 11(2):550-557 2010; Rawas-Qalaji et al. *Drug Dev Ind Pharm* 33(5):523-530 2007; Rawas-Qalaji et al. *AAPS PharmSci Tech* 7(2):Article 41 2006), have shown to be bioequivalent to epinephrine intramuscular injection in a validated rabbit model (Rawas-Qalaji et al. *J Allergy Clin Immunol*
10 117(2):398-403 2006; Rawas-Qalaji et al. *Biopharm Drug Dispos* 27(9):427-435 2006).

Incorporation of epinephrine nanoparticles fabricated using a high shear fluid processor (Microfluidizer) into the fast-disintegrating tablets, enhances the sublingual bioavailability of epinephrine.

15 Epinephrine was shown to be stable in these fast-disintegrating sublingual tablets for at least seven years. However, efforts should still be made to limit or minimize exposure of the tablets to oxygen and temperature.

The fast-disintegrating, taste-masked epinephrine sublingual tablets described herein are suitable for Phase I studies in humans and have the potential
20 to transform the treatment of anaphylaxis and other conditions responsive to epinephrine.

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. It is to be understood that while a certain form of the invention is illustrated, it is not
25 intended to be limited to the specific form or arrangement herein

described and shown. It will be apparent to those skilled in the art that various changes may be made without departing from the scope of the invention and the invention is not to be considered limited to what is shown and described in the specification. One skilled in the art will readily appreciate that the present invention

5 is well adapted to carry out the objectives and obtain the ends and advantages mentioned, as well as those inherent therein. The compositions, epinephrine nanoparticles, pharmaceutical tablets, methods, procedures, and techniques described herein are presently representative of the preferred embodiments, are intended to be exemplary and are not intended as limitations on the scope. Changes therein and

10 other uses will occur to those skilled in the art which are encompassed within the spirit of the invention. Although the invention has been described in connection with specific, preferred embodiments, it should be understood that the invention as ultimately claimed should not be unduly limited to such specific embodiments. Indeed various modifications of the described modes for carrying out the invention

15 which are obvious to those skilled in the art are intended to be within the scope of the invention.

CLAIMS

What is claimed is:

- 5 Claim 1. A pharmaceutical composition comprising at least an active ingredient having about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size of 230 ± 39 nm measured as a Z-average according to ISO 13321.
- 10 Claim 2. The pharmaceutical composition in accordance with claim 1, further comprising at least one non-medicinal ingredient, wherein the at least one non-medicinal ingredient is one of a diluent, a binder, a filler, a lubricant, a disintegrant, a penetration enhancer, a mucoadhesive, a taste enhancer, and a sweetening agent and mouthfeel enhancer.
- 15 Claim 3. The pharmaceutical composition in accordance with claim 2, wherein the pharmaceutical composition comprises at least three non-medicinal ingredients, the at least three non-medicinal ingredients comprising a filler, a lubricant, and a disintegrant.
- 20 Claim 4. The pharmaceutical composition in accordance with claim 3, wherein the filler is microcrystalline cellulose, the lubricant is magnesium stearate, and the disintegrant is a hydroxypropyl ether of cellulose.
- 25 Claim 5. The pharmaceutical composition in accordance with claim 3, further comprising at least one of a penetration enhancer, a mucoadhesive, a taste enhancer, and a sweetening agent and mouthfeel enhancer.
- 30 Claim 6. The pharmaceutical composition in accordance with claim 5, wherein the taste enhancer is citric acid and the sweetening agent and mouthfeel enhancer is mannitol.

Claim 7. The pharmaceutical composition in accordance with any one of claims 1-6, wherein the active ingredient is a pharmaceutically effective dose of about 40 mg stabilized nanoparticles of epinephrine bitartrate.

5 Claim 8. The pharmaceutical composition in accordance with any one of claims 1-7, wherein the pharmaceutical composition is formulated as a fast-disintegrating tablet for buccal or sublingual administration.

10 Claim 9. The pharmaceutical composition in accordance with claim 8, for increasing absorption of the buccally or sublingually administered epinephrine bitartrate upon disintegration of the fast-disintegrating tablet, wherein the increase in absorption results from formulation of the pharmaceutical composition with the stabilized nanoparticles of epinephrine bitartrate.

15 Claim 10. The pharmaceutical composition in accordance with claim 8, wherein the fast-disintegrating tablet is stable and remains pharmaceutically effective after a period of time ranging from about six months to about seven years.

20 Claim 11. A pharmaceutical composition formulated as a fast-disintegrating tablet, the pharmaceutical composition comprising:
an active ingredient having about 10 mg to about 40 mg stabilized nanoparticles of epinephrine bitartrate, the stabilized nanoparticles of epinephrine bitartrate having a mean size of 230 ± 39 nm measured as a Z-average according to ISO 13321 and having a coefficient of variation (RSD) of 17% at a concentration of 2.8 mg/ml in isopropyl alcohol
25 and at a pressure of 30,000 psi; and

a non-medicinal ingredient (NMI), wherein the non-medicinal ingredient is at least one of a diluent, a binder, a filler, a lubricant, a disintegrant, a penetration enhancer, a mucoadhesive, a taste enhancer, and a sweetening agent and mouthfeel enhancer.

30 Claim 12. The pharmaceutical composition in accordance with claim 1, wherein the stabilized nanoparticles of epinephrine bitartrate are prepared from a mixture of epinephrine bitartrate at a concentration of 2.8 mg/ml in an isopropyl alcohol solvent

exposed to one pass at a pressure of 30,000 psi and at the temperature in the range of 8.3 to 43.3 °C.

5 Claim 13. The pharmaceutical composition in accordance with claim 11, wherein the pharmaceutical composition comprises at least three non-medicinal ingredients, the at least three non-medicinal ingredients comprising a filler, a lubricant, and a disintegrant.

10 Claim 14. The pharmaceutical composition in accordance with claim 13, wherein the filler is microcrystalline cellulose, the lubricant is magnesium stearate, and the disintegrant is a hydroxypropyl ether of cellulose.

15 Claim 15. The pharmaceutical composition in accordance with claim 13, further comprising at least one of a penetration enhancer, a mucoadhesive, a taste enhancer, and a sweetening agent and mouthfeel enhancer.

20 Claim 16. The pharmaceutical composition in accordance with claim 15, wherein the taste enhancer is citric acid and the sweetening agent and mouthfeel enhancer is mannitol.

25 Claim 17. The pharmaceutical composition in accordance with claim 11, for use in increasing absorption of the buccally or sublingually administered epinephrine bitartrate upon disintegration of the fast-disintegrating tablet, wherein the increase in absorption results from formulation of the pharmaceutical composition with the stabilized nanoparticles of epinephrine bitartrate.

30 Claim 18. The pharmaceutical composition in accordance with claim 11, wherein the fast-disintegrating tablet is stable and remains pharmaceutically effective after a period of time ranging from about six months to about seven years.

 Claim 19. The pharmaceutical composition in accordance with any one of claims 11-17, wherein the active ingredient is a pharmaceutically effective dose of about 40 mg stabilized nanoparticles of epinephrine bitartrate.

Claim 20. The pharmaceutical composition in accordance with claim 1, wherein the stabilized nanoparticles of epinephrine bitartrate are non-encapsulated.

5 Claim 21. The pharmaceutical composition in accordance with claim 11, wherein the stabilized nanoparticles of epinephrine bitartrate are non-encapsulated.

Claim 22. The pharmaceutical composition in accordance with any one of claims 1-21, for use in increasing plasma concentration of epinephrine in a subject having
10 a condition responsive to epinephrine.

Claim 23. The pharmaceutical composition for use in accordance with claim 22, wherein the condition responsive to epinephrine is cardiac arrest or an allergic reaction.

15 Claim 24. The pharmaceutical composition for use in accordance with claim 23, wherein the allergic reaction is anaphylaxis, asthma, or bronchial asthma.

Claim 25. The pharmaceutical composition for use in accordance with claim
20 22, wherein the condition responsive to epinephrine is a breathing difficulty.

Claim 26. The pharmaceutical composition for use in accordance with claim 25, wherein the breathing difficulty is associated with anaphylaxis, asthma, bronchial asthma, bronchitis, emphysema, or a respiratory infection.

25 Claim 27. Use of the pharmaceutical composition in accordance with any one of claims 1-21, for increasing plasma concentration of epinephrine in a subject having a condition responsive to epinephrine.

30 Claim 28. The use in accordance with claim 27, wherein the condition responsive to epinephrine is cardiac arrest or an allergic reaction.

Claim 29. The use in accordance with claim 28, wherein the allergic reaction is anaphylaxis, asthma, or bronchial asthma.

Claim 30. The use in accordance with claim 27, wherein the condition
5 responsive to epinephrine is a breathing difficulty.

Claim 31. The use in accordance with claim 30, wherein the breathing difficulty is associated with anaphylaxis, asthma, bronchial asthma, bronchitis, emphysema, or a respiratory infection.

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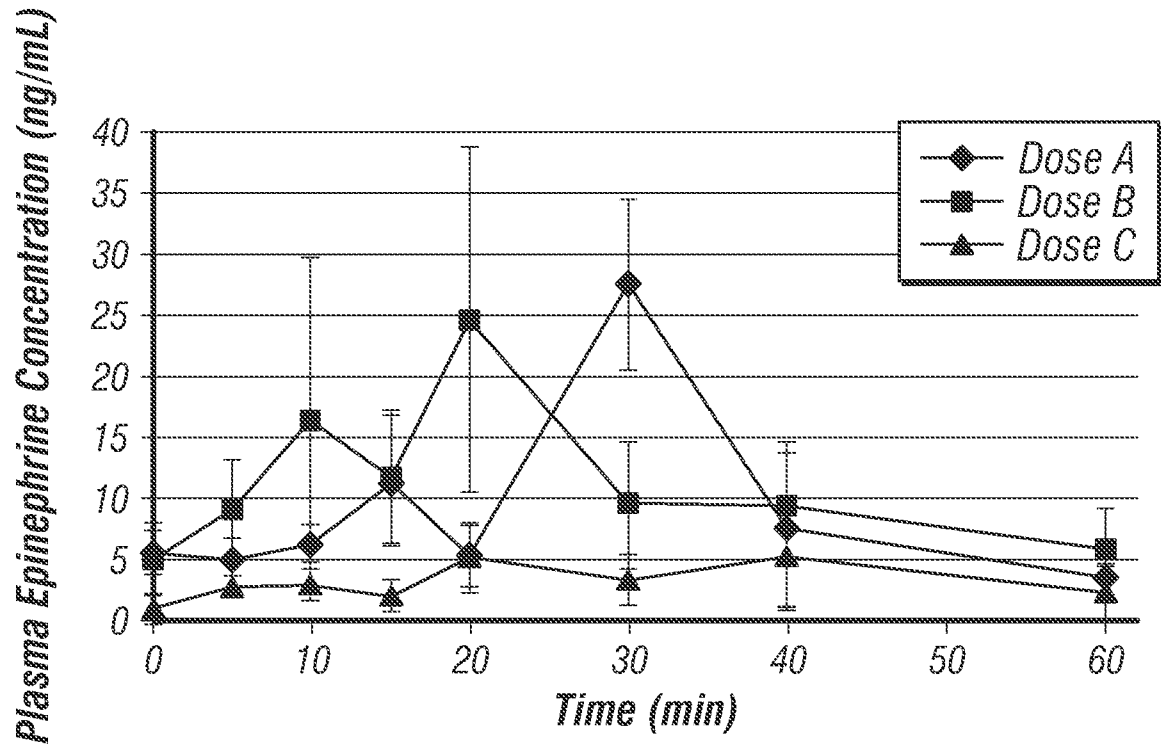


FIG. 1

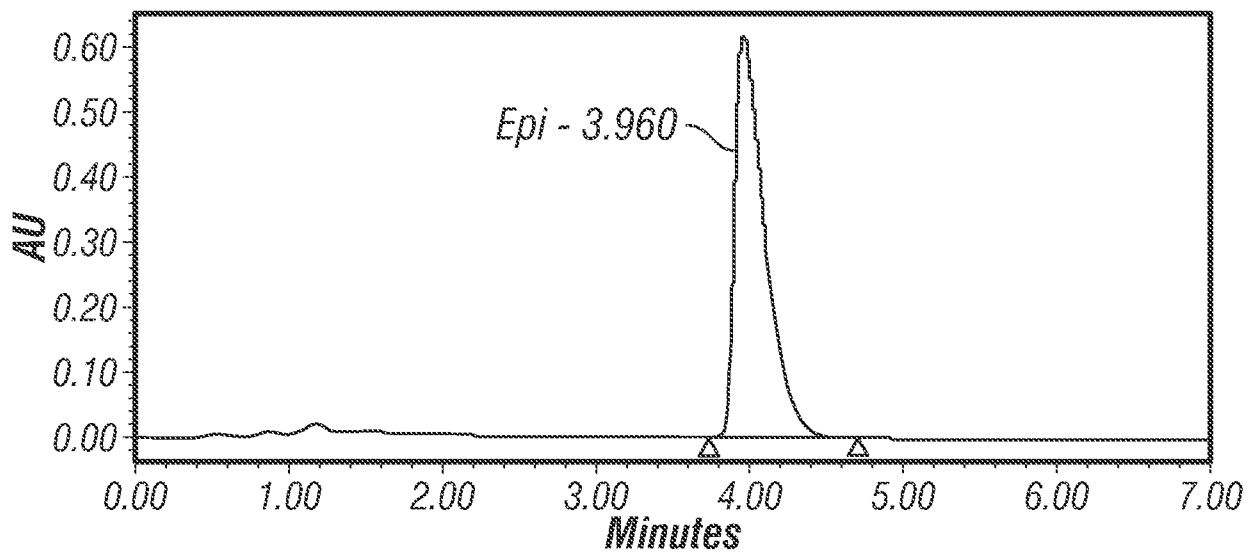


FIG. 2

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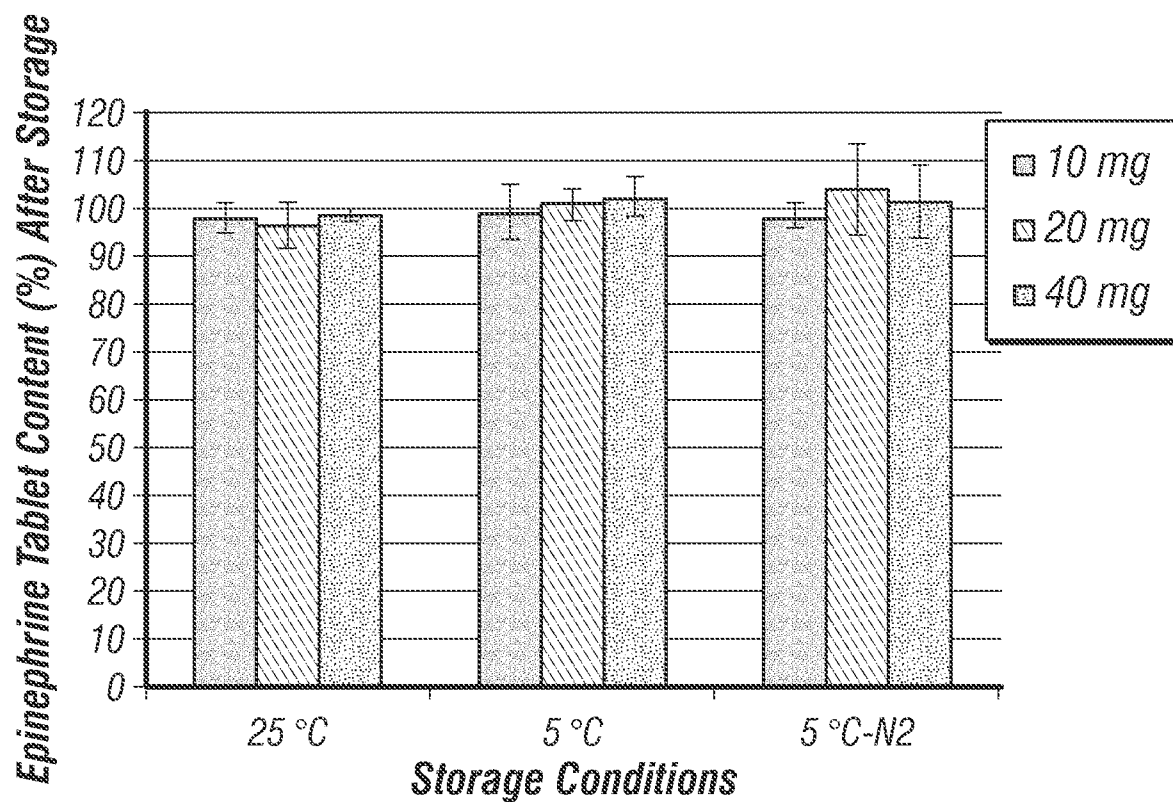


FIG. 3

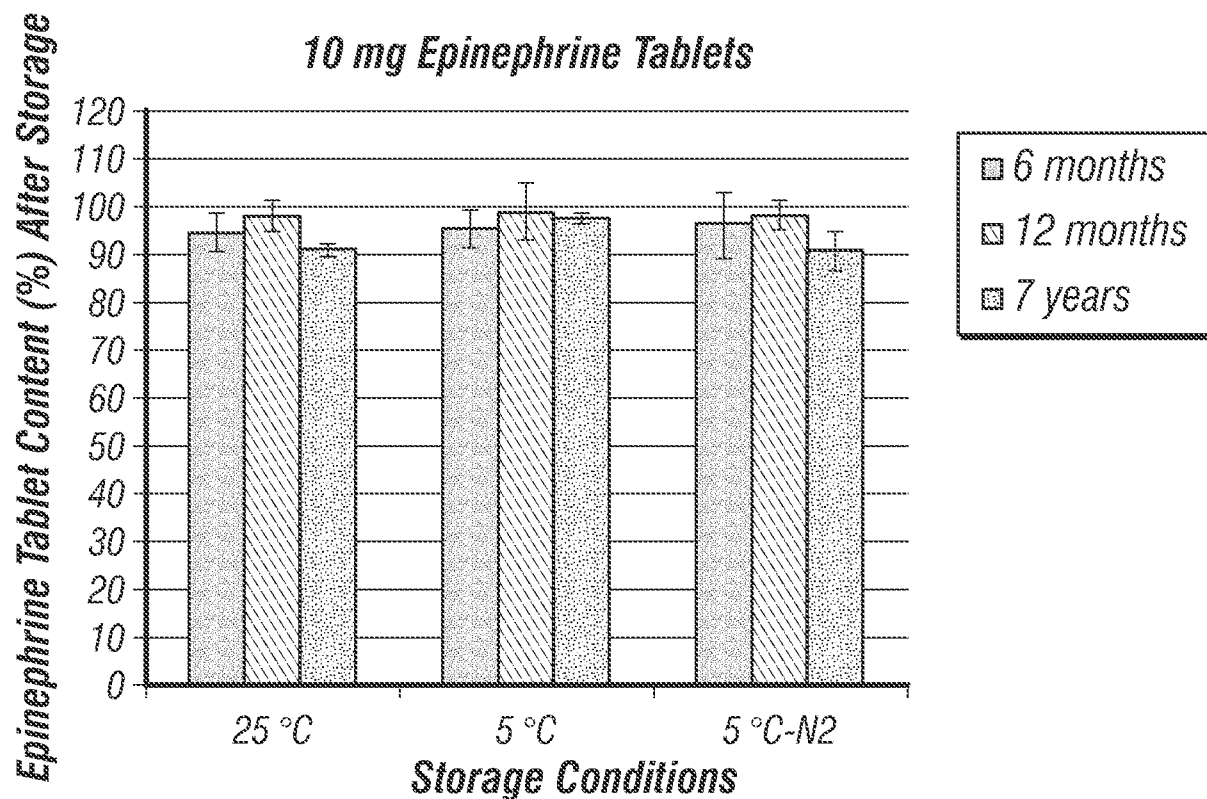


FIG. 4

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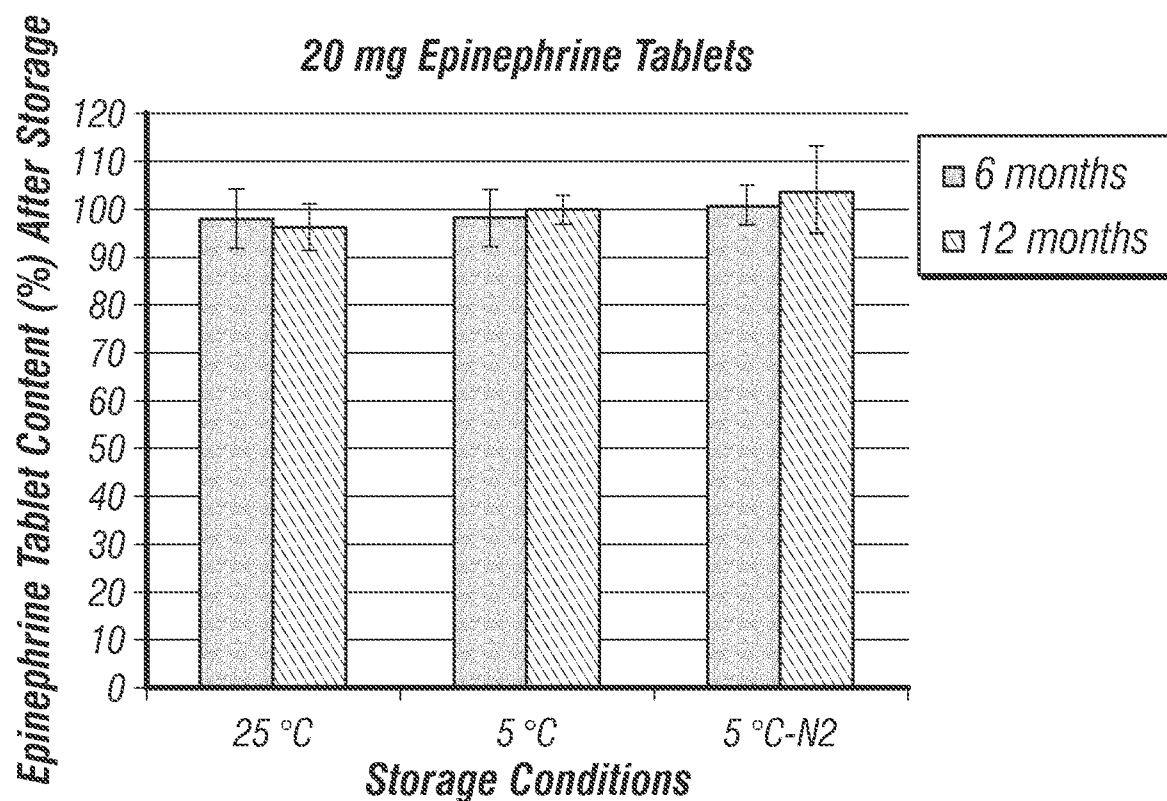


FIG. 5

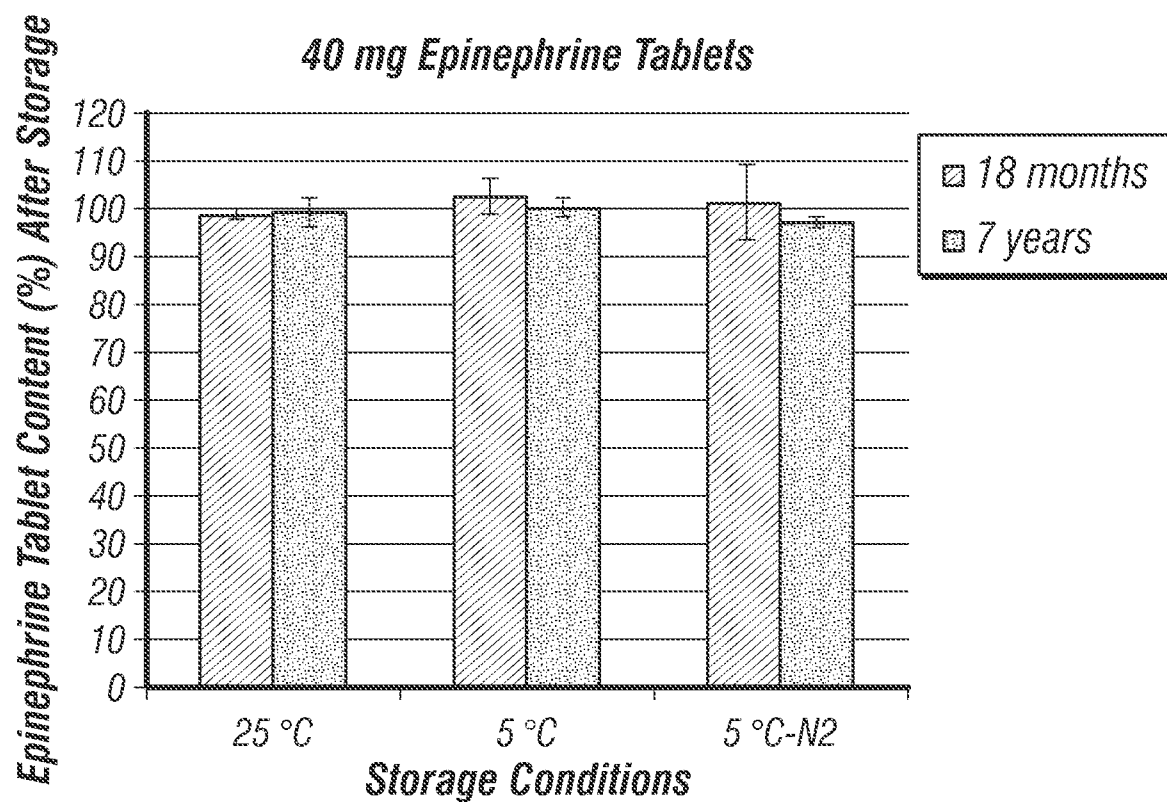


FIG. 6

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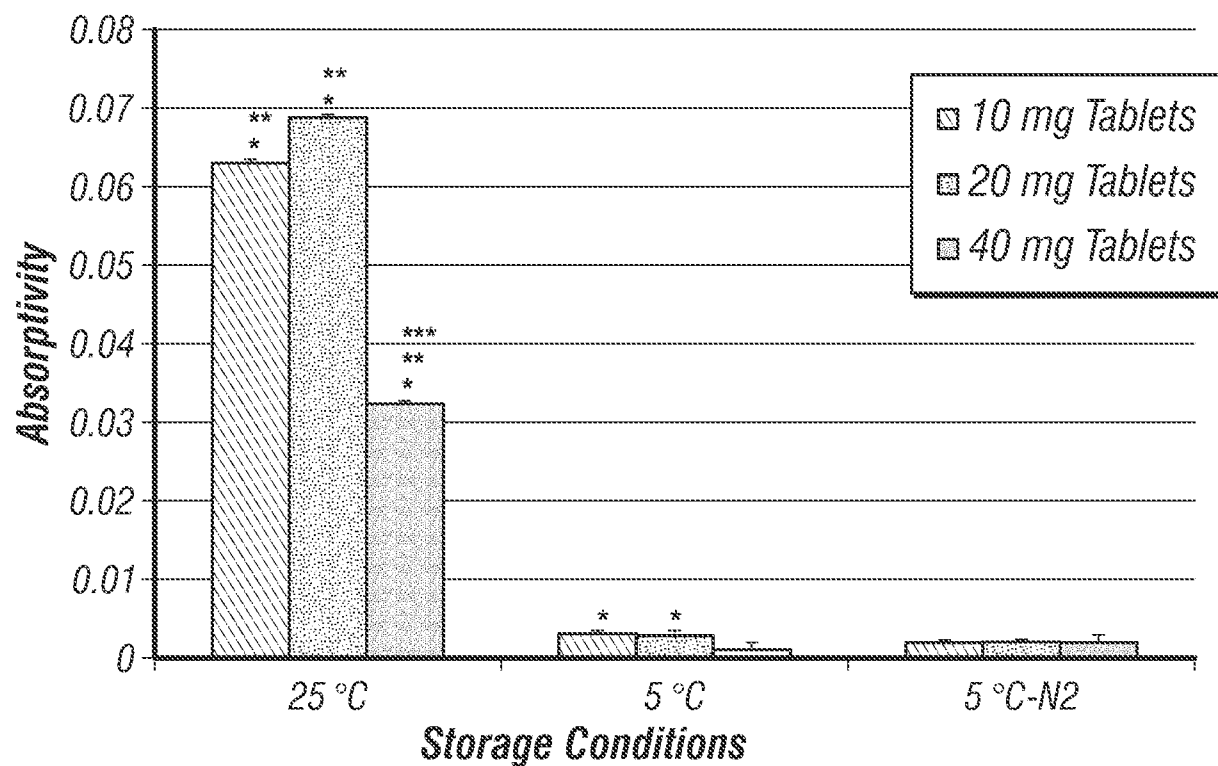


FIG. 7

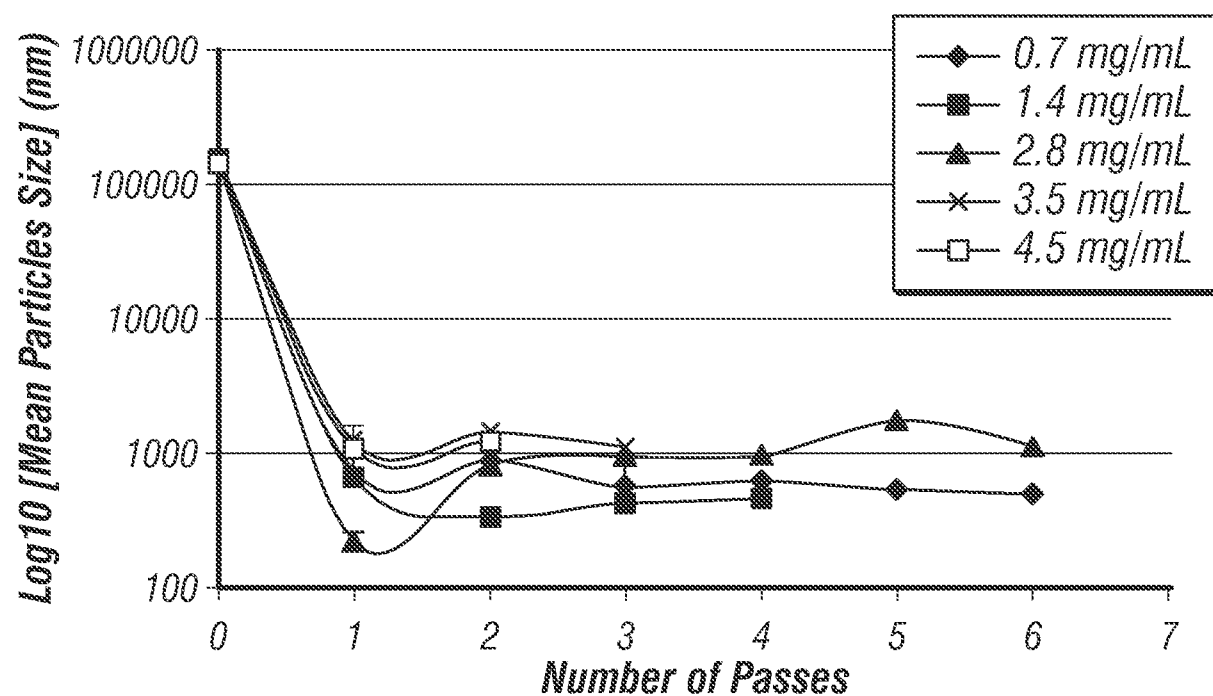
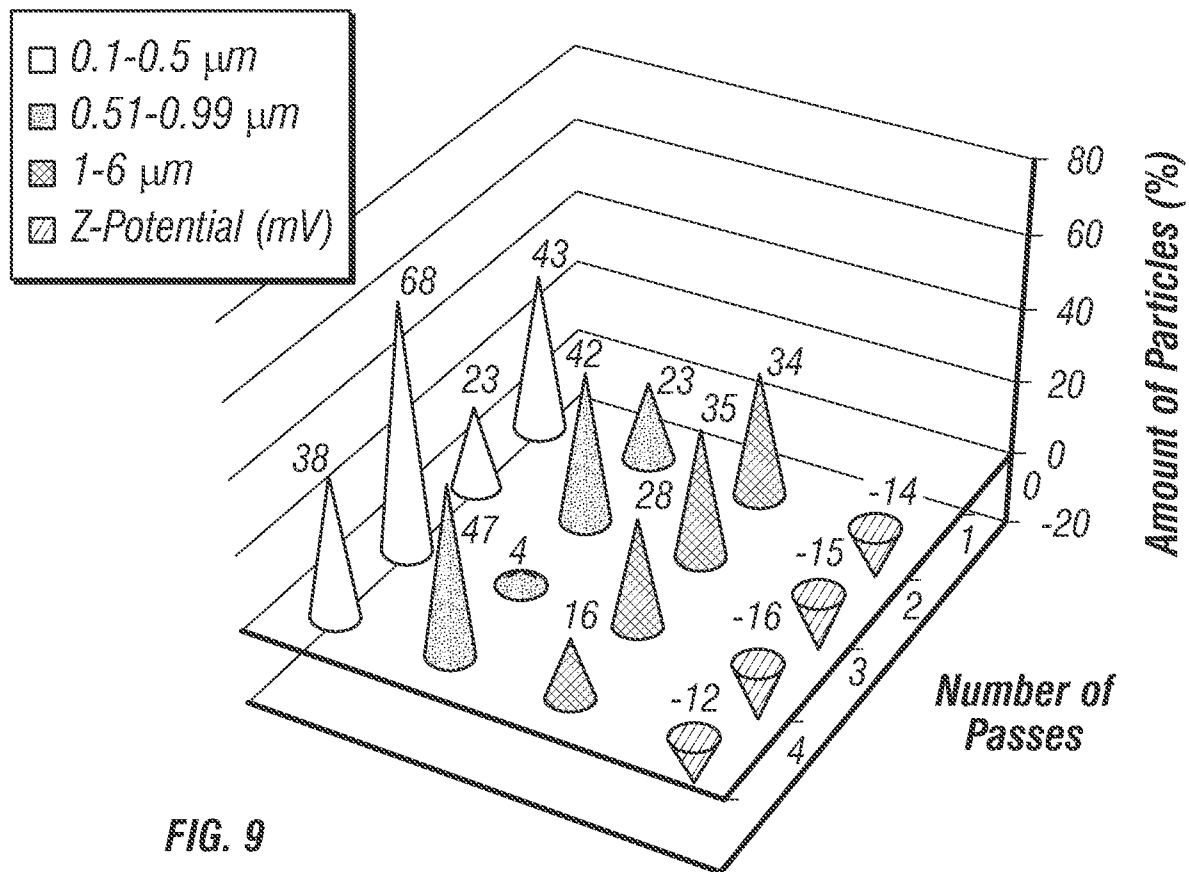
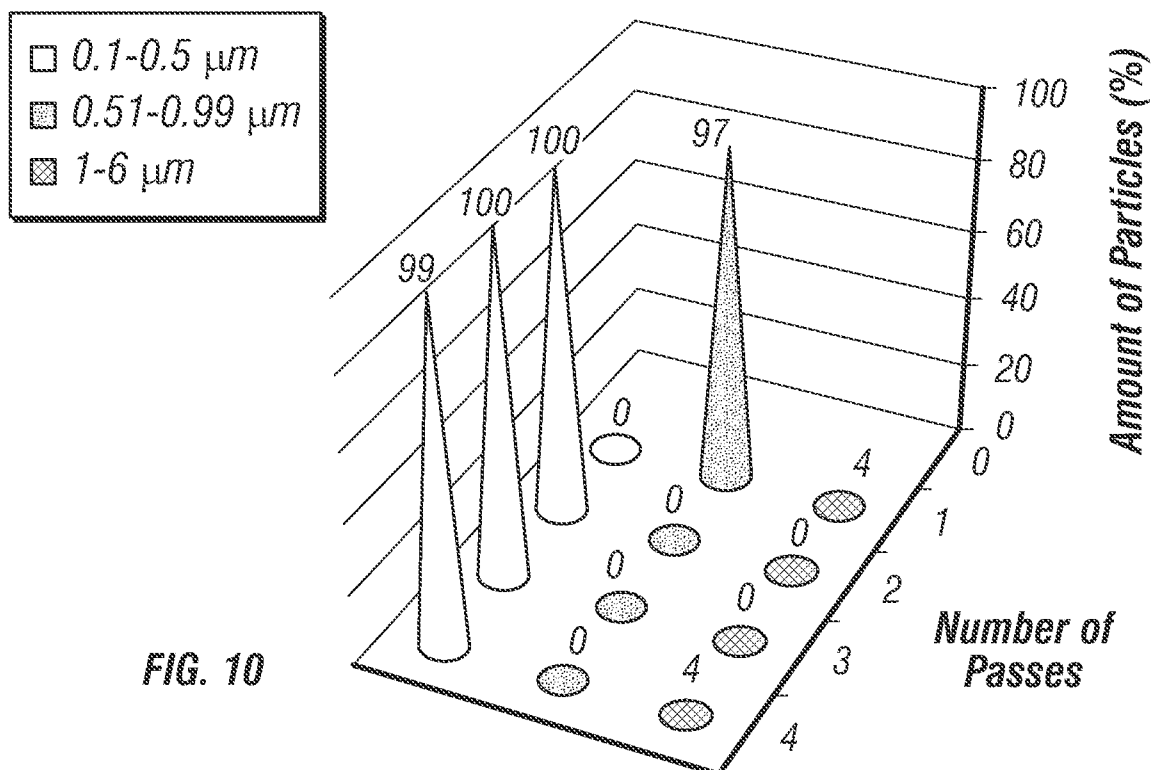


FIG. 8

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0.7 mg/mL Epinephrine Bitartrate in IPA**1.4 mg/mL Epinephrine Bitartrate in IPA**

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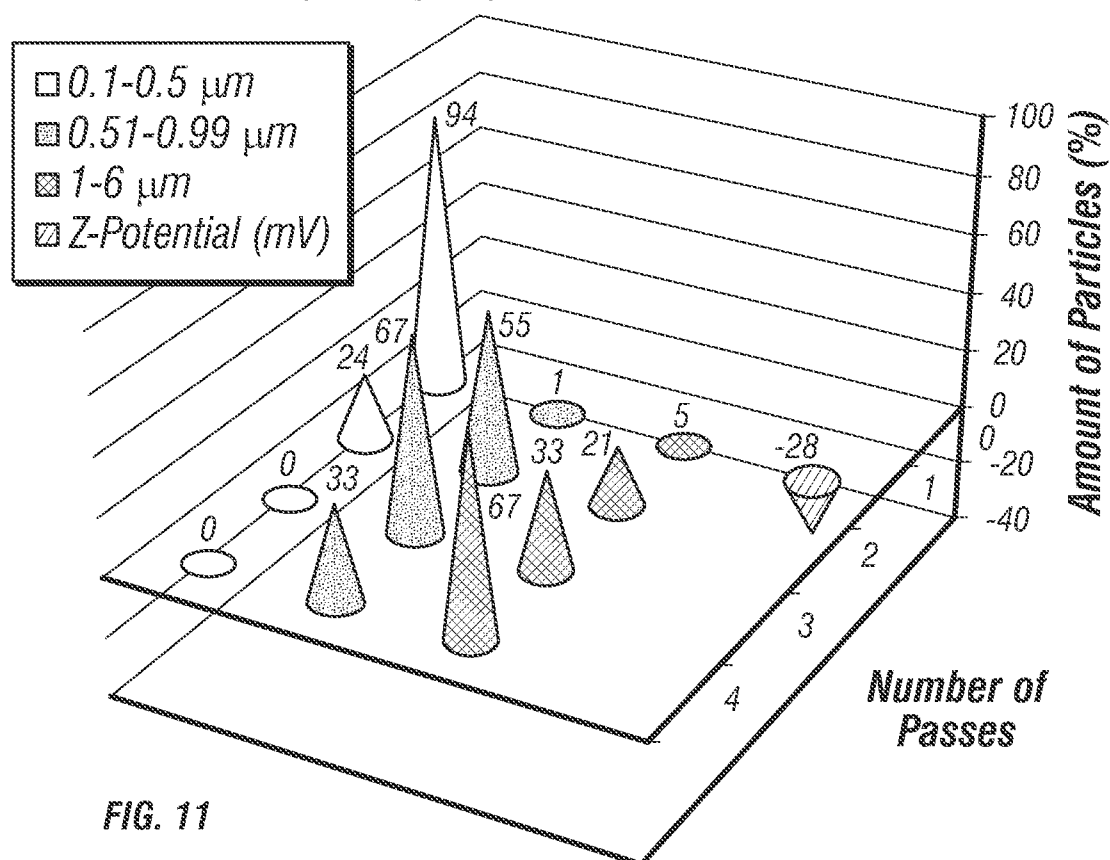
2.8 mg/mL Epinephrine Bitartrate in IPA

FIG. 11

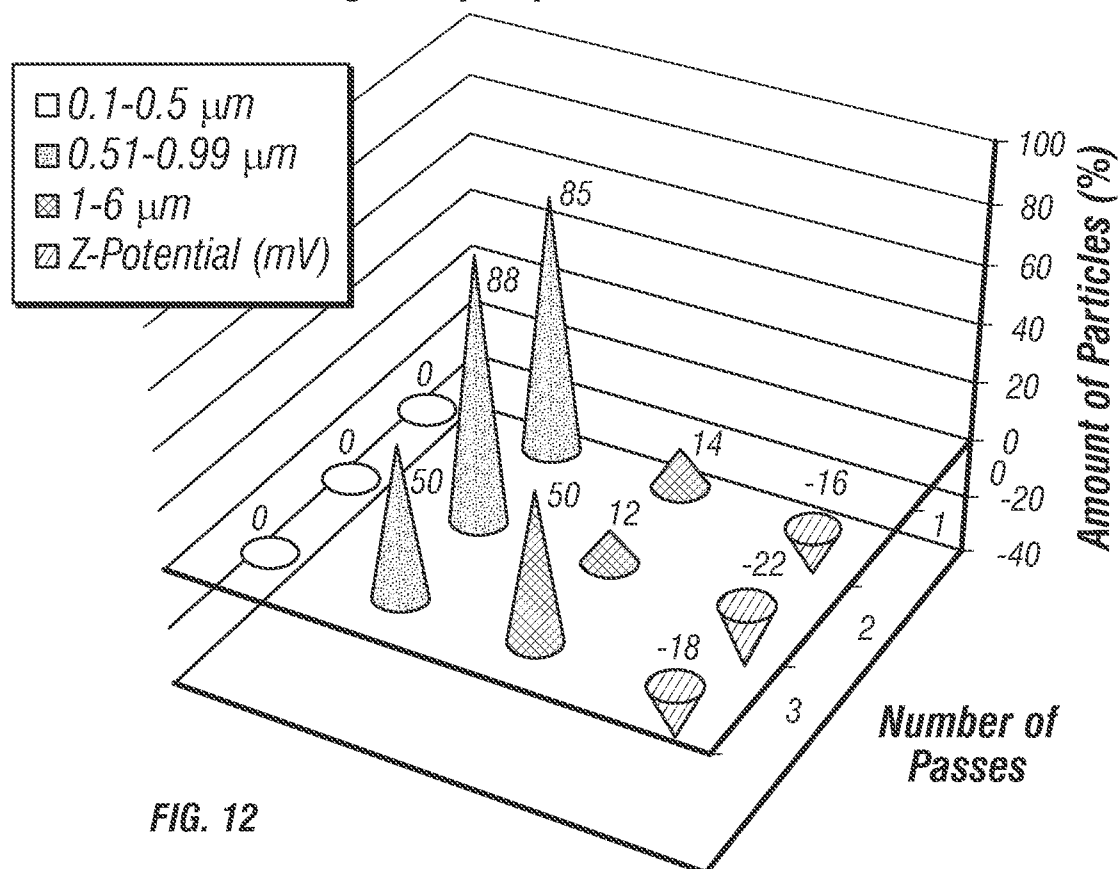
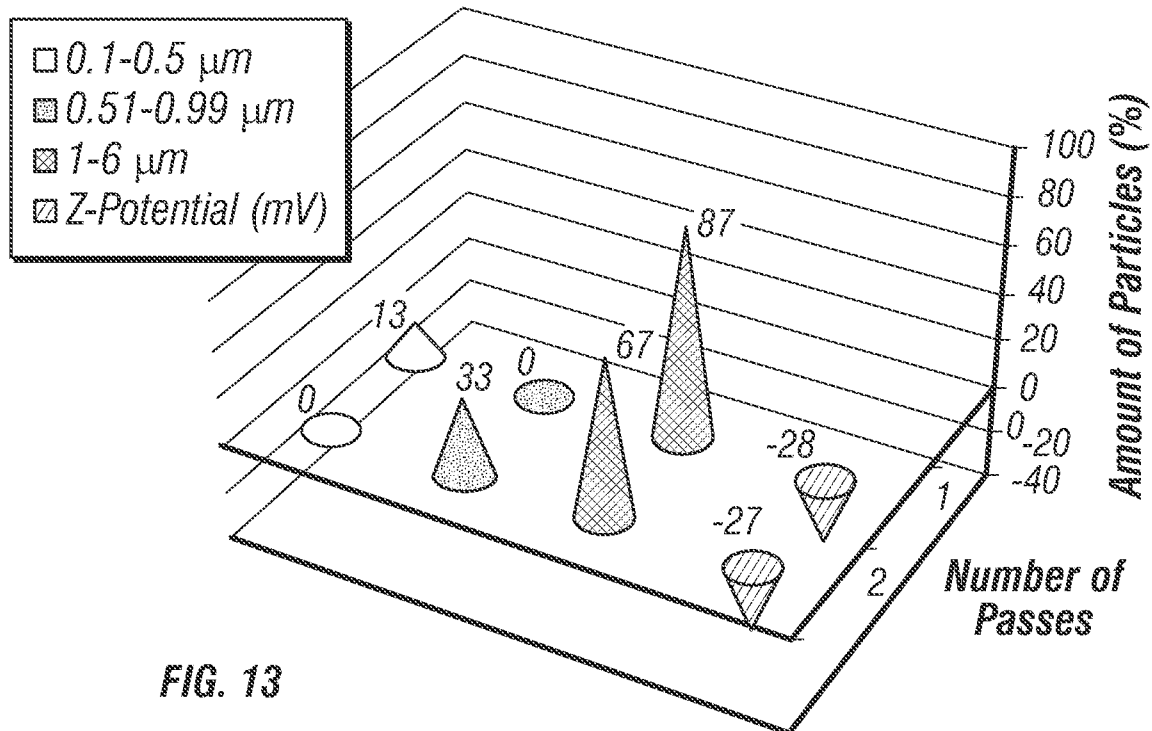
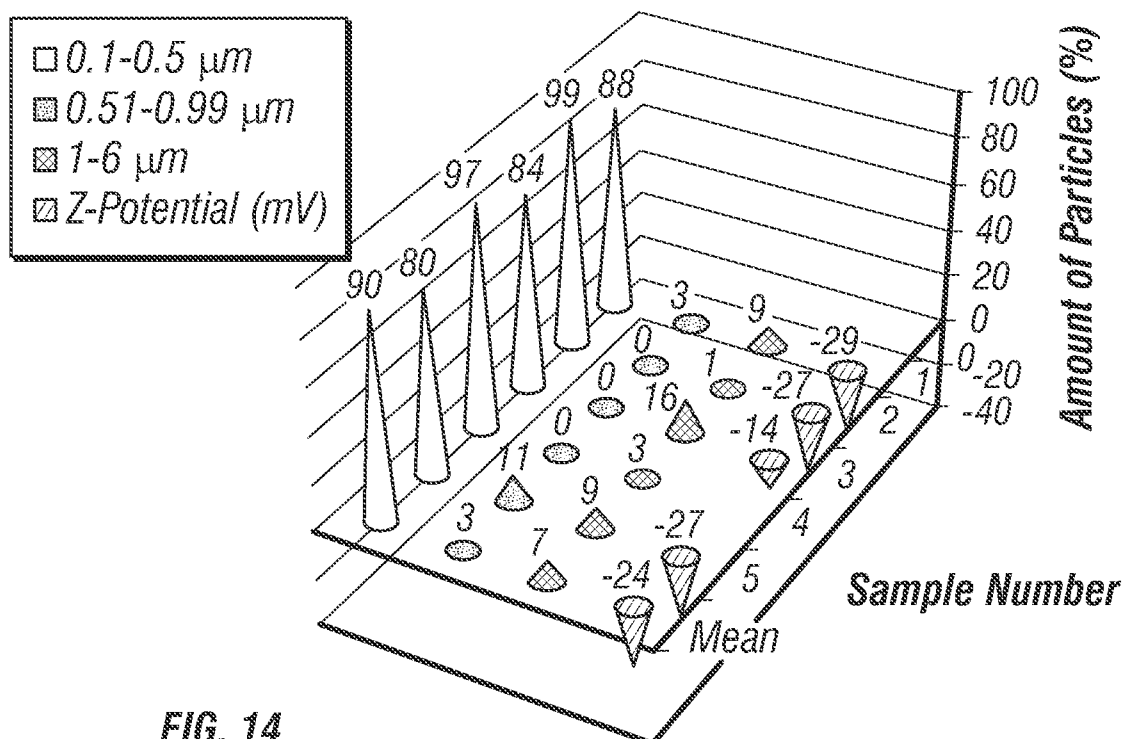
3.5 mg/mL Epinephrine Bitartrate in IPA

FIG. 12

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4.5 mg/mL Epinephrine Bitartrate in IPA**FIG. 13****Reproducibility of Processing 2.8 mg/mL Epinephrine Bitartrate in IPA****FIG. 14**

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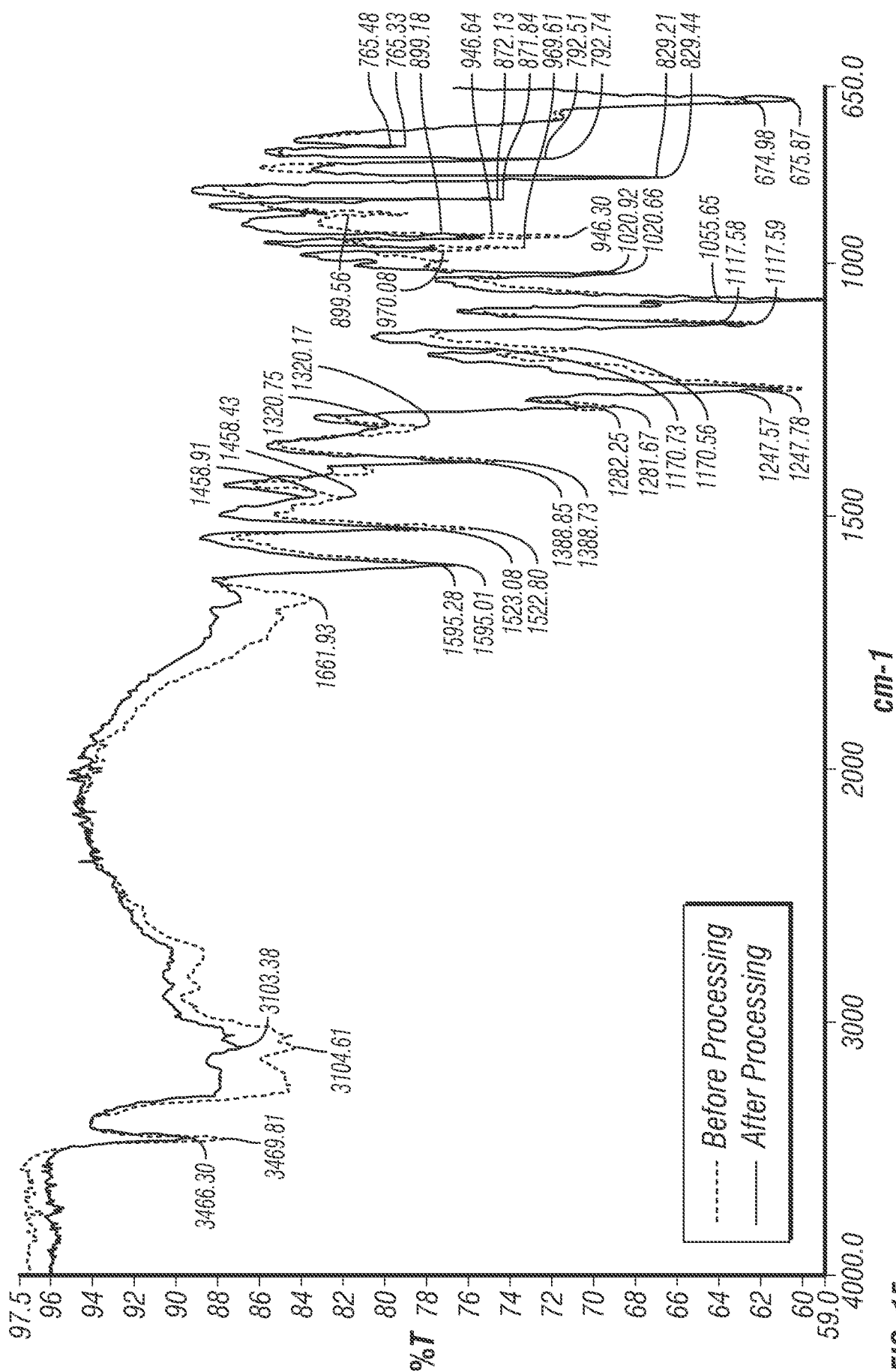


FIG. 15

Plasma Epinephrine Concentration (ng/mL)

